

CHAPTER IV

RESULTS



RESULTS

Objective 1: To evaluate the effect of cannabis extract on albino mice

4.1.1 Body weight measurement

The effects of three different concentrations of cannabis extract on albino mice's body weight are shown in table 2. The results revealed that body weight was significantly ($P=0.05$) varied among the treatment groups at 2nd week. The highest value was T3 ($33.85\pm1.11g$) significantly varied from the lowest value T1 ($26.63\pm1.02g$). There were no significant ($p<0.05$) variations observed between T0 and T1, T2 and T3. In the 1st and 2nd weeks of observation ($p<0.05$) significant variations were observed among the treatment groups. The highest body weight was observed at T3 ($40.90\pm1.18g$) in 3rd week of observation significantly varied ($p<0.05$) to the lowest value at T0 ($28.35\pm0.87g$).

Table 2: Effects of three different concentrations of cannabis extract on body weight gain in albino mice.

Time interval	Treatment groups				P-value
	T0 (g)	T1 (g)	T2 (g)	T3 (g)	
1 st week	24.85 ± 0.64^a	24.58 ± 0.87^a	27.88 ± 0.39^b	29.09 ± 0.69^c	0.02
2 nd week	26.78 ± 0.75^a	26.63 ± 1.02^a	32.73 ± 1.71^b	33.85 ± 1.11^b	0.05
3 rd week	28.35 ± 0.87^a	28.10 ± 0.82^a	38.17 ± 3.28^b	40.90 ± 1.18^b	0.01

The values are Mean $\pm$ SE, ^{abc} values having different superscripts in the same row differed significantly. T0, T1, T2, and T3 indicate the control, 5mg/kg, 10mg/kg, and 15mg/kg by body weight of mice. SE=Standard error of the mean, p-value= probability value.

4.1.2 Biochemical profile

4.1.2.1 RBS

The effects of three different concentrations of cannabis extract on RBS in albino mice are presented in table 3. In the 1st and 2nd weeks of observations, the RBS result differed significantly ($P<0.05$) among the different treatment groups of mice except in the 3rd week after treatment with cannabis extract. In the 1st week after treatment, mice of the T3

group ($3.20 \pm 0.15 \text{ mmolL}^{-1}$) had significantly ($P < 0.05$) lower RBS than T0 ($5.56 \pm 0.78 \text{ mmolL}^{-1}$) and T1 ($5.70 \pm 0.62 \text{ mmolL}^{-1}$) group but there was no significant difference was found between T2 and T3 treatment groups. After 2nd week of injection, RBS was significantly ($P < 0.05$) lowest in the T3 group ($3.43 \pm 0.67 \text{ mmolL}^{-1}$) compared to T1 ($4.17 \pm 0.17 \text{ mmolL}^{-1}$), T2 ($4.27 \pm 0.27 \text{ mmolL}^{-1}$) and T0 ($5.27 \pm 0.15 \text{ mmolL}^{-1}$) respectively.

Table 3: Effects of three different concentrations of cannabis extract on RBS in albino mice

Time interval	Treatment groups				P-value
	T0 (mmolL^{-1})	T1 (mmolL^{-1})	T2 (mmolL^{-1})	T3 (mmolL^{-1})	
1 st week	5.56 ± 0.78^b	5.70 ± 0.62^b	3.63 ± 0.19^a	3.20 ± 0.15^a	0.01
2 nd week	5.27 ± 0.15^c	4.17 ± 0.17^a	4.27 ± 0.27^b	3.43 ± 0.67^a	0.03
3 rd week	5.40 ± 0.35	5.10 ± 1.00	3.37 ± 0.37	3.80 ± 0.41	0.12

The values are Mean $\pm$ SE, ^{abc} values having different superscripts in the same row differed significantly. T0, T1, T2, and T3 indicate the control, 5mg/kg, 10mg/kg, and 15mg/kg by body weight of mice treatment groups. SE=Standard error of the mean, p-value= probability value.

4.1.2.2 Serum Creatinine

The effects of three different concentrations of cannabis extract on serum creatinine results in albino mice are shown in table no. 4. In the 2nd week of mice observations serum creatinine results varied significantly ($p < 0.05$) compare to the other treatment groups of mice. There was no significant variation among the treatment groups in the 1st week and 2nd week of observation. In the 2nd week after extract injection, mice of T3 ($0.17 \pm 0.03 \text{ mg/dl}$) has significantly ($P < 0.05$) lower serum creatinine results than T1 ($0.37 \pm 0.07 \text{ mg/dl}$). In 3rd week of the observation group, no ($p < 0.05$) significant differences were observed among the treatment groups of T0, T1, T2, and T3 respectively.

Table 4: Effects of three different concentrations of cannabis extract on Serum creatinine in albino mice

Time interval	Treatment groups				P-value
	T0 (mg/dl)	T1 (mg/dl)	T2 (mg/dl)	T3 (mg/dl)	
1 st week	0.23±0.07	0.17±0.63	0.20±0.11	0.16±0.06	0.76
2 nd week	0.20±0.01 ^a	0.37±0.07 ^b	0.27±0.03 ^{ab}	0.17±0.03 ^a	0.04
3 rd week	0.27±0.09	0.37±0.03	0.23±0.03	0.27±0.03	0.47

The values are Mean±SE and ^{abcd} values having different superscripts differed significantly. T0, T1, T2, and T3 indicate the control, 5mg/kg, 10mg/kg, and 15mg/kg by body weight of mice treatment groups. SE=Standard error of the mean, p-value= probability value.

4.1.2.3 SGPT

The effects of three different concentrations of cannabis extract on SGPT/ALT results in albino mice are shown in table 5. SGPT results differed significantly ($P<0.05$) among the different treatment groups of mice in the 1st, 2nd and 3rd week of observation. In the 1st week of observation, mice of T0 (20.67±1.86U/L) had significantly ($P<0.05$) highest SGPT result was T3 (674±217.22U/L) but there was no significant difference was found in T1 and T2 group. In the 2nd week of observation, mice of T0 (23.33±1.85U/L) had significantly ($p<0.05$) the highest SGPT result at T1 (458.33±235.34). In the 3rd week of observation, mice T0 (32.67±0.88) had significantly ($p<0.05$) highest at T1 (675.33±407.95) but no significant variation between T2 and T3.

Table 5: Effects of three different concentrations of cannabis extract on SGPT in albino mice

Time interval	Treatment groups				P-value
	T0 (U/L)	T1 (U/L)	T2 (U/L)	T3 (U/L)	
1 st week	20.67±1.86 ^a	300.67±86.61 ^b	327.66±61.49 ^b	674±27.22 ^c	0.03
2 nd week	23.33±1.85 ^a	458.33±35.34 ^d	243.66±38.29 ^b	331.67±37.76 ^c	0.02
3 rd week	32.67±0.88 ^a	675.33±47.95 ^c	172.67±30.03 ^b	102.33±66.90 ^b	0.01

The values are Mean±SE, ^{abc} values having different superscripts in the same row differed significantly. T0, T1, T2, and T3 indicate the control, 5mg/kg, 10mg/kg, and 15mg/kg by body weight of mice treatment groups. SE=Standard error of the mean, p-value= probability value.

4.1.2.4 SGOT

The effects of three different concentrations of cannabis extract on SGOT/AST test results in albino mice are shown in table no. 6. The 1st week of observation shows a significant variation ($p<0.05$) compare to the other treatment groups. The significantly highest value was observed at T3 (674.67 ± 217.95), and no significant variation ($p<0.05$) was observed between T0 and T2 groups. There were no significant variations ($p<0.05$) in the 2nd week and 3rd weeks of observation among the treatment groups.

Table 6: Effects of three different concentrations of cannabis extract on SGOT in albino mice

Time interval	Treatment groups				P-value
	T0 (U/L)	T1 (U/L)	T2 (U/L)	T3 (U/L)	
1 st week	30.00 ± 7.50^a	157.67 ± 81.20^b	44.00 ± 37.24^a	674.67 ± 217.95^c	0.01
2 nd week	19.67 ± 1.45	458.33 ± 235.34	200.00 ± 85.05	436.00 ± 67.42	0.13
3 rd week	28.00 ± 2.52	675.21 ± 211.31	314.67 ± 116.83	562.67 ± 155.21	0.33

The values are Mean $\pm$ SE, ^{abc} values having different superscripts in the same row differed significantly. T0, T1, T2, and T3 indicate the control, 5mg/kg, 10mg/kg, and 15mg/kg by body weight of mice treatment groups. SE=Standard error of the mean, P-value= probability value.

4.1.2.5 Alkaline Phosphatase

The effects of cannabis extract on alkaline phosphatase are shown in table no. 7. ($P<0.05$) significant variation of alkaline phosphatase was found among the treatment groups of mice. In the 1st week of cannabis extract injection, the highest results of alkaline phosphatase observed at T3 (982.67 ± 276.83) were significantly varied among the T0 and T1 treatment groups of mice. In the 2nd week of observation, the results show ($p=0.05$) significant variation among the treatment groups. The highest value at T3 (508.33 ± 168.66 U/L) of alkaline phosphatase was significantly varied ($p=0.05$) to the treatment groups of T0 and T2.

Table 7: Effects of three different concentrations of cannabis extract on serum alkaline phosphatase in albino mice.

Time interval	Treatment groups				P-value
	T0 (U/L)	T1 (U/L)	T2 (U/L)	T3 (U/L)	
1 st week	100.67±6.17 ^a	310.00±30.32 ^b	495.67±192.32 ^{bc}	982.67±276.83 ^c	0.03
2 nd week	102.33±7.22 ^a	559.00±270.95 ^c	202.67±76.39 ^b	508.33±168.66 ^c	0.05
3 rd week	87.33±6.74	152.33±47.67	347.00±177.25	231.33±32.40	0.30

The values are Mean±SE, ^{abc} values having different superscripts in the same row differed significantly. T0, T1, T2, and T3 indicate the control, 5mg/kg, 10mg/kg, and 15mg/kg by body weight of mice treatment groups. SE=Standard error of the mean, p-value= probability value.

4.1.2.6 Serum Cholesterol

The effects of cannabis extract on serum cholesterol are given in table 8. There was no significant ($p < 0.05$) difference found among all treatment groups of mice at 1st week, 2nd week, and 3rd week of mice cannabis extract injection. The highest result of serum cholesterol was at T3 (170.33±32.77mg/dl) in the 2nd week and the lowest result was at T2 (100.33±17.07mg/dl) in the 3rd week of observation.

Table 8: Effects of three different concentrations of cannabis extract on Serum cholesterol in albino mice.

Time interval	Treatment groups				P-value
	T0 (mg/dl)	T1 (mg/dl)	T2 (mg/dl)	T3 (mg/dl)	
1 st week	130.33±10.33	119.00±9.53	107.00±18.50	164.00±22.87	0.16
2 nd week	134.00±1.53	116.67±13.38	139.33±28.29	170.33±32.77	0.45
3 rd week	137.33±3.38	150.33±14.90	100.33±17.07	152.33±30.33	0.26

The values are Mean±SE. T0, T1, T2, and T3 indicate the control, 5mg/kg, 10mg/kg, and 15mg/kg by body weight of mice treatment groups. SE=Standard error of the mean, p-value= probability value.

4.1.2.7 Serum Triglyceride

The results of serum triglyceride are shown in table no. 9. In the 1st and 2nd weeks of observations, serum triglyceride results differed significantly ($P < 0.05$) among the different treatment groups of mice except for the 3rd week after treatment with cannabis extract. In the 1st week after treatment, mice of T0 (94.67 ± 12.17 mg/dl) had significantly ($P < 0.05$) lower serum triglyceride than T3 (155.33 ± 20.79 mg/dl) and T2 (139.00 ± 12.34 mg/dl) group but there was no significant difference was found in T1 treatment groups. In the 2nd week after treatment, mice of T0 (96.00 ± 13.85 mg/dl) were significantly ($p < 0.05$) different from the treatment group of T2 (109.33 ± 14.84 mg/dl) and T3 (161.33 ± 16.55 mg/dl). But there were no significant variations observed among the treatment groups of T0 and T1.

Table 9: Effects of three different concentrations of cannabis extract on serum triglyceride in albino mice.

Time interval	Treatment groups				P-value
	T0 (mg/dl)	T1 (mg/dl)	T2 (mg/dl)	T3 (mg/dl)	
1 st week	94.67 ± 12.17^a	81.00 ± 10.26^a	139.00 ± 12.34^b	155.33 ± 20.79^c	0.02
2 nd week	96.00 ± 13.85^a	91.67 ± 9.70^a	109.33 ± 14.84^b	161.33 ± 16.55^c	0.03
3 rd week	102.00 ± 16.70	98.00 ± 13.00	121.33 ± 10.67	149.33 ± 10.17	0.08

The values are Mean $\pm$ SE, ^{abc} values having different superscripts in the same row differed significantly. T0, T1, T2, and T3 indicate the control, 5mg/kg, 10mg/kg, and 15mg/kg by body weight of mice treatment groups. SE=Standard error of the mean, p-value= probability value.

4.1.2.8 Uric acid

The effects of three different concentrations of cannabis extract on uric acid test results in albino mice are given in table no. 10. The serum uric acid result significantly ($p < 0.05$) differed among the treatment group in 1st week, but no significant ($p < 0.05$) variation was observed at 2nd, and 3rd week of cannabis extract injection groups. In the 1st week of the treatment group, the highest uric acid test result at T3 (30.37 ± 5.60 mg/dl) significantly varied ($p < 0.05$) to the T0, T1, and T3 treatment groups of mice.

Table 10: Effects of three different concentrations of cannabis extract on uric acid in albino mice.

Time interval	Treatment groups				P-value
	T0 (mg/dl)	T1 (mg/dl)	T2 (mg/dl)	T3 (mg/dl)	
1 st week	5.93±0.59 ^a	25.43±7.54 ^{bc}	13.13±3.40 ^b	30.37±5.60 ^c	0.03
2 nd week	7.00±0.17	18.57±4.77	15.07±4.39	20.67±6.69	0.25
3 rd week	5.87±1.39	17.20±5.35	10.70±4.39	15.37±6.9	0.42

The values are Mean±SE, ^{abc} values having different superscripts in the same row differed significantly. T0, T1, T2, and T3 indicate the control, 5mg/kg, 10mg/kg, and 15mg/kg by body weight of mice treatment groups. SE=Standard error of the mean, p-value= probability value.

4.1.3 Hematological profile

4.1.3.1 Hemoglobin

The effects of three different concentrations of cannabis extract on hemoglobin in albino mice are shown in table no. 11. There were no significant results ($p < 0.05$) observed in any group of mice. The highest result of hemoglobin (14.97±0.03g/dl) in the 1st week of T1 treatment, and the lowest result of hemoglobin (13.60±0.60 g/dl) in the 3rd week T2 treatment group of mice. No significant variation ($p < 0.05$) was observed among any treatment groups in the 1st, 2nd, and 3rd week respectively.

Table 11: Effects of three different concentrations of cannabis extract on hemoglobin in albino mice.

Time interval	Treatment groups				P-value
	T0 (g/dl)	T1 (g/dl)	T2 (g/dl)	T3 (g/dl)	
1 st week	14.13±0.61	14.97±0.03	14.63±0.31	13.97±0.57	0.43
2 nd week	14.26±0.63	14.10±0.66	14.03±0.61	13.93±0.52	0.98
3 rd week	14.20±0.15	14.47±0.47	13.60±0.60	14.50±0.29	0.43

The values are Mean±SE. T0, T1, T2, and T3 indicate the control, 5mg/kg, 10mg/kg, and 15mg/kg by body weight of mice treatment groups. SE=Standard error of the mean, NS= non-significant, p-value= probability value.

4.1.3.2 RBC count

The effects of three different concentrations of cannabis extract on RBC in albino mice are shown in table no. 12. There were no significant results ($p < 0.05$) observed in any treatment group of mice. The highest result of RBC ($7.86 \pm 0.34 \times 10^6/\text{cmm}$) in the 2nd week of T3 treatment, and the lowest result of hemoglobin ($4.93 \pm 1.44 \times 10^6/\text{cmm}$) in the 1st week T2 treatment group of mice. No significant variation was observed among any treatment groups in the 1st, 2nd, and 3rd week respectively.

Table 12: Effects of three different concentrations of cannabis extract on RBC in albino mice.

Time interval	Treatment groups				P-value
	T0 ($10^6/\text{cmm}$)	T1 ($10^6/\text{cmm}$)	T2 ($10^6/\text{cmm}$)	T3 ($10^6/\text{cmm}$)	
1 st week	7.2 ± 0.84	6.1 ± 1.37	4.93 ± 1.44	7.27 ± 0.42	0.45
2 nd week	6.50 ± 0.85	7.33 ± 1.62	5.33 ± 1.77	7.86 ± 0.34	0.56
3 rd week	6.47 ± 0.79	5.01 ± 1.67	6.63 ± 1.48	7.34 ± 0.34	0.62

The values are Mean $\pm$ SE. T0, T1, T2, and T3 indicate the control, 5mg/kg, 10mg/kg, and 15mg/kg by body weight of mice treatment groups. SE=Standard error of the mean, NS= non-significant, p-value= probability value.

4.1.3.3 WBC count

The effect of cannabis extract-injected mice on WBC count test results is shown in table 13. There were no significant ($p < 0.05$) results observed compared to all treatment groups of mice. The highest result of WBC count was ($4.10 \pm 0.61/\text{cmm}$) in the 2nd week T0 treatment group and the lowest result of WBC count was ($2.65 \pm 0.69/\text{cmm}$) in the 1st week T1 treatment group of mice.

Table 13: Effects of three different concentrations of cannabis extract on white blood cell count in albino mice.

Time interval	Treatment groups				P-value
	T0 (10 ³ /cmm)	T1 (10 ³ /cmm)	T2 (10 ³ /cmm)	T3 (10 ³ /cmm)	
1 st week	3.48±0.95	2.65±0.69	3.40±0.45	3.87±0.77	0.79
2 nd week	4.10±0.61	2.91±0.28	3.2±0.16	3.82±2.08	0.21
3 rd week	3.40±0.30	2.99±0.64	3.72±0.42	3.72±0.41	0.54

The values are Mean±SE. T0, T1, T2, and T3 indicate the control, 5mg/kg, 10mg/kg, and 15mg/kg by body weight of mice treatment groups. SE=Standard error of the mean, NS= non-significant, p-value= probability value.

4.1.3.4 Platelet Count

The effects of three different concentrations of cannabis extract on platelet count test results in albino mice are shown in table no. 14. No significant ($p < 0.05$) variation was observed compared to all treatment groups of mice at the 1st, 2nd and 3rd week of cannabis extract injection. The highest results of platelet count were (6.17±1.64) in the 3rd week T0 treatment group and the lowest results of platelet count were (3.52±0.311) in the 2nd week T0 treatment group of mice. No significant variation was observed among any treatment groups at the 1st, 2nd, and 3rd week of cannabis extract treatment respectively.

Table 14: Effects of three different concentrations of cannabis extract on platelet counts in albino mice.

Time interval	Treatment groups				P-value
	T0 (10 ⁵ /cmm)	T1 (10 ⁵ /cmm)	T2 (10 ⁵ /cmm)	T3 (10 ⁵ /cmm)	
1 st week	3.65±0.60	4.42±0.82	6.19±1.29	4.62±1.03	0.37
2 nd week	3.52±0.311	5.55±1.23	5.82±1.15	4.67±0.91	0.41
3 rd week	6.17±1.64	5.46±0.60	5.91±0.83	5.12±0.87	0.90

The values are Mean±SE. T0, T1, T2, and T3 indicate the control, 5mg/kg, 10mg/kg, and 15mg/kg by body weight of mice treatment groups. SE=Standard error of the mean, NS= non-significant, p-value= probability value.

4.1.4 Identification of cannabinoid by GC-MS

The figure shows the GC-MS of cannabis extract using ethanol as a solvent. 2ml ethanol extract had used for GC-MS.

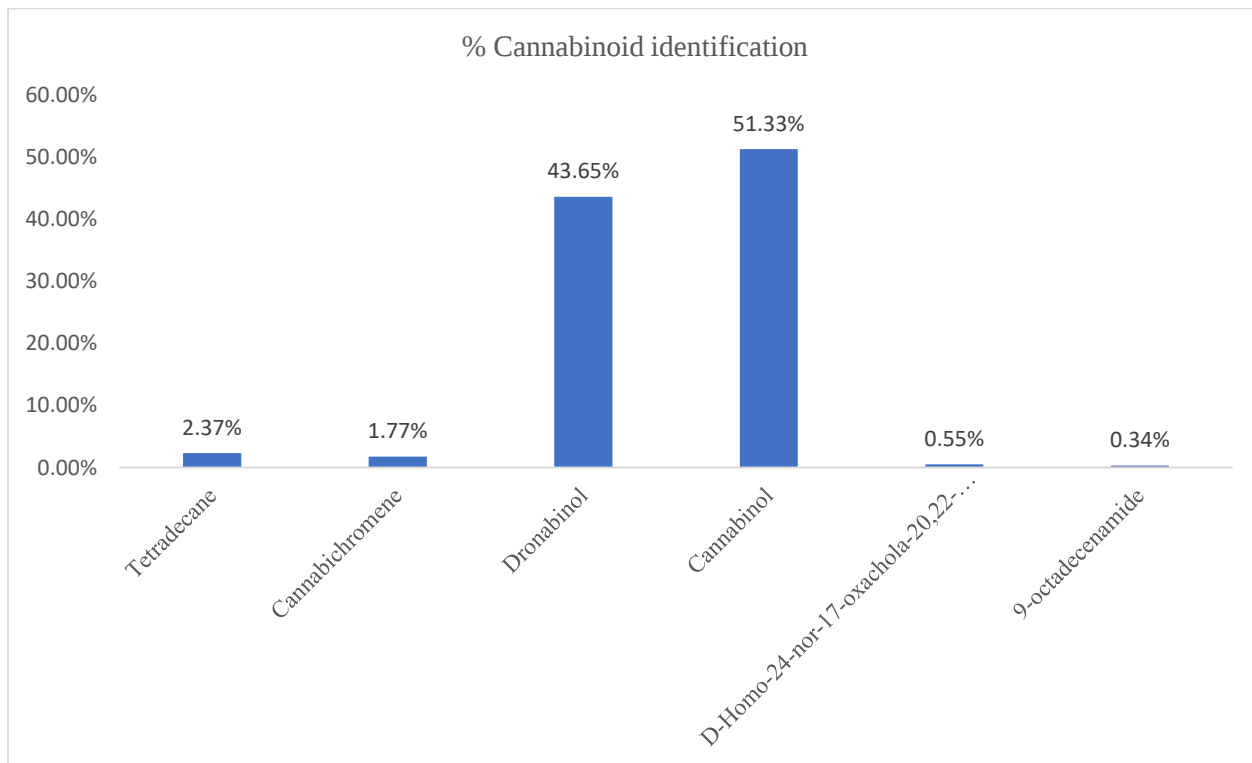


Figure 35: Quantitative result of *C. sativa* ethanol extract

4.1.5 Acute toxicity study

Four different doses of cannabis extract had used in this study. 10mg/kg, 20mg/kg, 40mg/kg and 80mg/kg doses of extract had used. In this case, at 40mg/kg, only 3 mice survived from 6 mice. 10mg/kg and 20mg/kg doses injected mice all were alive at 24h of observation. LD50 measures 40mg/kg in 20-25g (average body weight) of albino mice.

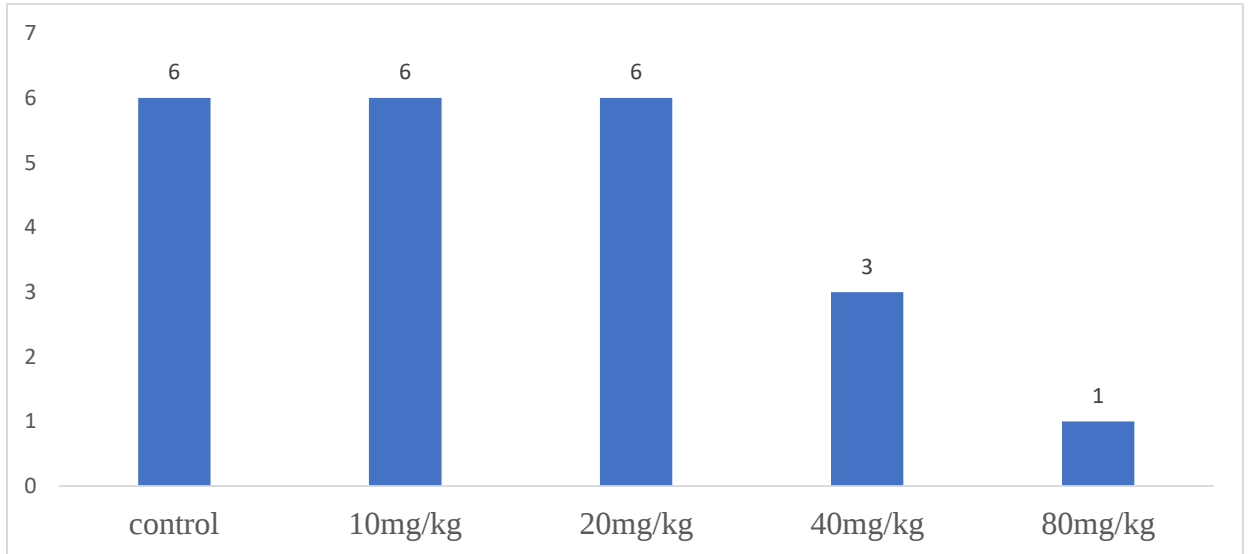


Figure 36: Acute toxicity study of cannabis extract on albino mice

4.1.6 Hematological study

Hematological slides of both the normal and extract-treated mice were similar in WBC count.

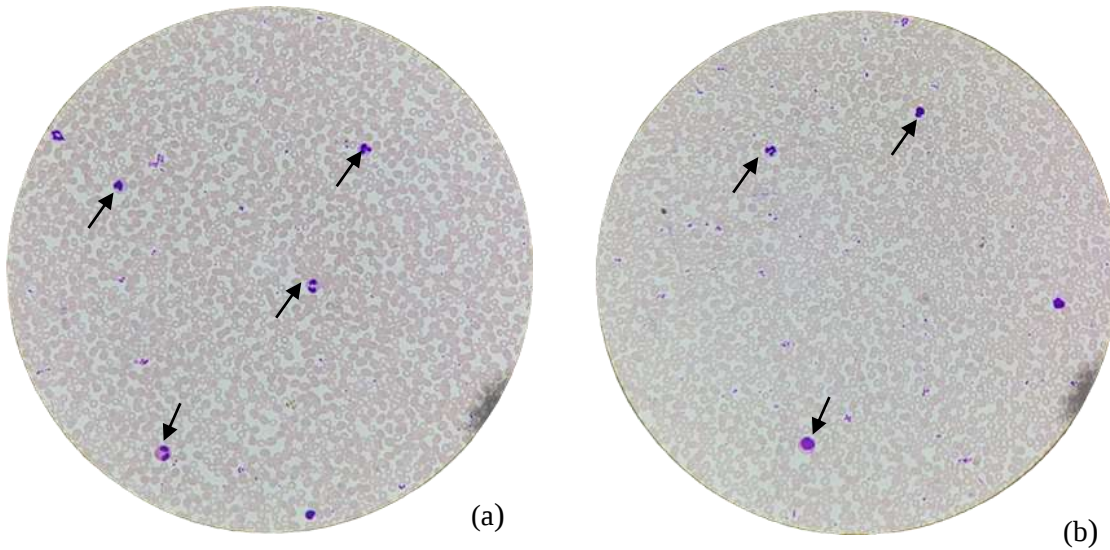


Figure 37: The image shows the effects of three different concentrations of cannabis extract on WBC count in albino mice. The image shows the differences between (a) normal mice and (b) cannabis extract inject mice hematological slide, using a light microscope 400X view.

4.1.7 Histopathological study

The main organ (such as the liver, kidney, heart, pancreas, and lung) of mice were collected when mice were sacrificed. The marking area of (a) shows inflammatory cells on the lung and (b) shows hepatocellular damage in the liver. For another organ lung, heart, kidney and pancreas no cellular changes were observed. For other organs such as the lungs, kidney, pancreas, and heart no kind of cellular changes were found.

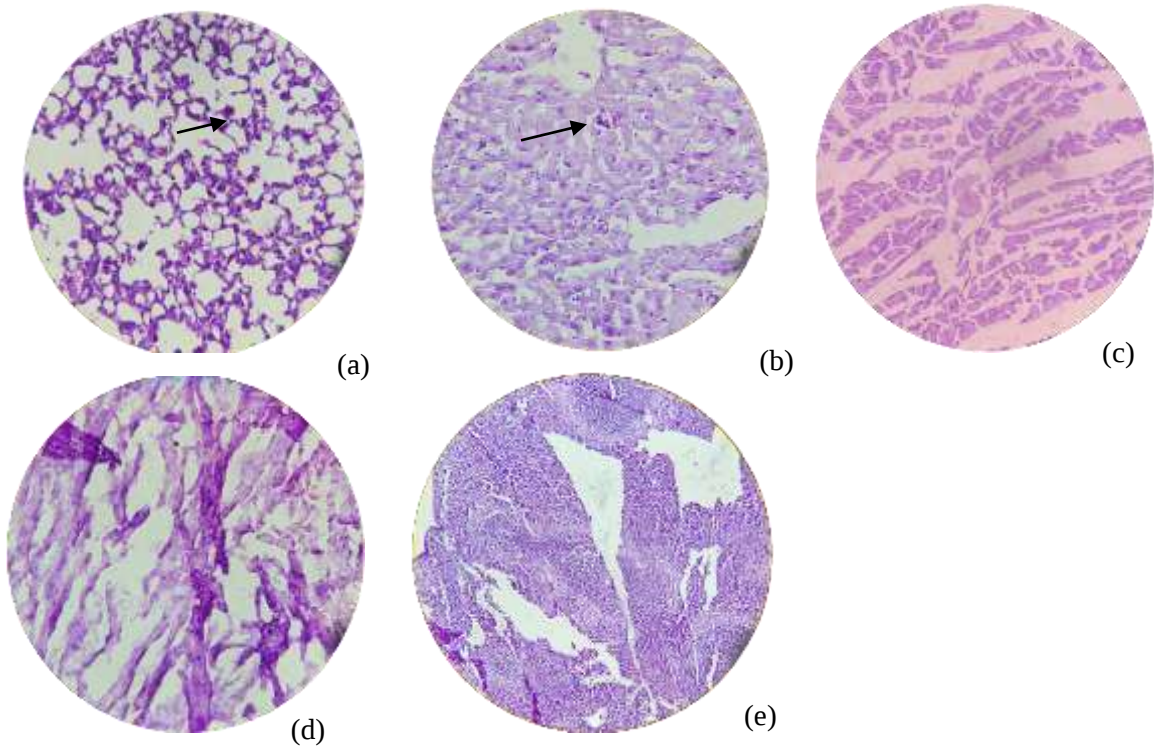


Figure 38: The images show the effects of three different concentrations of cannabis extract on cellular changes in albino mice. Mice main organ histopathological observation using H and E dyes and 400X view under a light microscope, a=lung, b=liver, c=heart, d=kidney, and e=pancreas.