

4.1. Phytochemical Analysis of Aqueous *Aloe vera* Gel Extract

Qualitative analysis of phytochemicals was conducted on the aqueous extract of *Aloe vera* gel revealed the presence of tannins, carbohydrates and anthraquinones, However, alkaloids were found to be absent in the extract. Various plant extracts are used for the treatment of various ailments because of the presence of these phytochemicals which have been reported to have medicinal values and contribute towards its antioxidant properties [Table 5.1].

4.1.1. Antioxidant Activity of Aqueous *Aloe vera* Gel Extract

ABTS and DPPH radical scavenging activity of aqueous *Aloe vera* gel extracts was shown in [Figure 5.1(a) and 5.1 (b)]. ABTS assay is used for determining the antioxidant activity of hydrogen-donating antioxidants. The *Aloe vera* extract efficiently scavenged ABTS radicals generated by the reaction between ABTS and ammonium persulfate was found to be increased in dose-dependent manner. DPPH is a stable free radical frequently used to determine radical scavenging activity of natural compounds. The extract showed potent radical scavenging activity in concentration dependent manner in both ABTS and DPPH assay. However, *Aloe vera* gel extract exhibited good radical scavenging activity when compared to BHT and tannic acid as standards. Free radical scavenging activity increased with increasing concentration of aqueous *Aloe vera* gel extract. The results showed that the IC₅₀ value of extract in ABTS assay was found to be 20 µg/µl and in DPPH assay IC₅₀ value of extract was 50 µg/µl.

4.1.2. Quantitative Estimation of Various Components of Aqueous *Aloe vera* Gel Extract

The quantitative estimation of primary metabolites reveals various chemical constituents present in the plant [Table 5.2]. In the present study it is found that *Aloe vera* extract contains carbohydrates, flavanoids and protein contents being responsible for its marked antioxidant activity as assayed through various *in vitro* models. Flavonoids are regarded as one of the most widespread groups of natural constituents found in the plants. It has been recognized that flavonoids show antioxidant activity through scavenging or chelating process. Carbohydrates are the major source of energy fuels for metabolic processes readily assimilable though fats yield more energy. It is responsible for imparting the moisturizing effect to the gel. Total carbohydrate content was found to be 7.33 µg/mg wet weight of *Aloe vera* gel extract followed by protein (4.08 µg/ mg wet weight of *Aloe vera* gel extract) and the amount was calculated from the regression equation of calibration curve ($y = 0.0342 x$, $r^2 = 0.996$) and is expressed as glucose equivalents. Total flavonoid content was estimated by aluminium chloride method using quercetin as standard. The amount was calculated from the regression equation of calibration curve ($y = 0.0098 x$, $r^2 = 0.996$) and is expressed as quercetin equivalents. The amount of total flavonoids content was found to be 4.28 µg/ mg weight of *Aloe vera* gel extract.

4.1.3. Trace Elements in Aqueous *Aloe vera* Gel Extract

Minerals are nutritive elements which are present in tissues and fluids of all body. They maintain the certain physio-chemical processes, structural components of tissues and as constituents of enzymes in many metabolic pathways. The quantitative analysis of various trace elements like K, Zn, Cu, Fe, Mn, Cl, Mg, Na, Ru, Mo, Br, Pd, Rd, Ni etc. in *Aloe vera* extract was determined by using WD-XRF technique. The highest concentration of K, Mg, Ca and Na were recorded in *Aloe vera* gel extract. The concentration of other elements analysed in the present study were found to be decreased in the order Fe > Al > Mn > Zn > Sr > Cu > Br > Rb > Cr > Ni > Zr [Table 5.3]. Each element has their individual role in maintain the structural and functional integrity of cells of living organisms.

4.2. Standardization of Aqueous *Aloe vera* Gel Extract Dose

Prior to administration, safe dose of *Aloe vera* gel extract was standardized. For this various doses of aqueous *Aloe vera* gel extract (250 mg/ kg b.w., 100 mg/ kg b.w. and 50 mg/ kg b.w.) was fed daily to the animals for 30 days. At the end of the study, control and treated animals were then sacrificed and various organs were excised to check for histopathological alterations, LPO level and LDH activity in serum and various tissues.

4.2.1. Effect of Various Doses of Aqueous *Aloe vera* Gel Extract on Body Weight, Diet Intake and Water Consumption

After the completion of treatment with various doses of *Aloe vera* gel extract (250, 100 and 50 mg/ kg b.w.) animals were examined for alterations in body

weight, diet intake and water consumption during the experimental period. Body weight, diet intake and water consumption remained unaltered in all the treatment groups throughout the treatment period.

4.2.2. Histopathology of Liver

Histopathological analysis of liver from control animal revealed normal histoarchitecture comprising of innumerable lobules consisting of a vast inter anastomosing network with single cell thick plates of hepatocytes separated by vascular sinusoids along with vascular channels radiating out from central veins, each of which is a hexagonal structure surrounded by portal areas containing hepatic triad with a branch of hepatic artery, a small bile duct, hepatic portal vein in control group [Figure 5.4 (a), (b)].

Aqueous *Aloe vera* gel extract treated animals (250 and 100 mg/ kg b.w.) [Figure 5.4 (c), (d), 5.4 (e), (f)] revealed many pathological alterations including inflammatory cell infiltration around hepatic portal vein, cell degeneration, enlargement of central vein. Aqueous *Aloe vera* gel extract treated animals (50 mg/ kg b.w.) [Figure 5.4 (g), (h)] revealed normal architecture.

4.2.3. Histopathology of Spleen

Histopathological analysis of spleen from control animal revealed normal architecture [Figure 5.5 (a), (b)] which included normal splenic architecture consisting of areas of white pulp with germinal center and surrounding lighter marginal zone, and red pulp. White pulp contains lymphoid aggregations,

mostly lymphocytes and macrophages which are arranged around the arteries. Red pulp has lots of vascular sinuses. There are connective tissues termed as trabeculae.

Aqueous *Aloe vera* gel extract treated animals (250 mg/ kg b.w. and 100 mg/ kg b.w.) [Figure 5.5 (c-f)] revealed widespread infiltration in white pulp, decreased white pulp, increased red pulp, dilated sinusoids. Aqueous *Aloe vera* gel extract treated animals (50 mg/ kg b.w.) [Figure 5.5 (g), (h)] revealed normal architecture with normal areas of white pulp along with germinal center and surrounding lighter marginal zone, and red pulp.

4.2.4. Histopathology of Kidney

Histopathological examination of kidney from control animals revealed normal histoarchitecture [Figure 5.6 (a), (b)], [Figure 5.7 (a), (b)]. A gross section of kidney corpuscle revealed two distinguished regions i.e. outer cortex and inner medulla. Outer cortex consist of a tuft of capillaries lined with epithelial cells, proximal and distal convoluted tubule (PCT and DCT) which are enclosed within a fibrous capsule called Bowman's capsule known as Renal corpuscle. The thick and thin parts of loop of Henle and greater part of collecting ducts are present in inner medulla comprised of renal pyramids.

Histopathological examination of kidney from animals treated with *Aloe vera* extract (250 and 100 mg/ kg b.w.) [Figure 5.6 (c-f)] [Figure 5.7 (c-f)] resulted in alterations in renal histoarchitecture which included atrophy of renal corpuscle, decrease in glomerular cellularity, reduction in number of Bowman's capsule, glomerular congestion with increased Bowman's spaces,

crowding of nuclei with hyperplastic response in inner medulla was observed. Aqueous *Aloe vera* gel extract treated animals (50 mg/ kg b.w.) [Figure 5.6 (g), (h)] [Figure 5.7 (g), (h)] resulted normal cortical labyrinth and medullary region.

4.2.5. Histopathology of Testes

Histopathological analysis of testes from control animals revealed normal architecture [Figure 5.8 (a), (b)] which included lumen of seminiferous tubules with normal spermatogonia which are in direct contact with epithelial basal lamina, diploid primary spermatocytes, spermatids with sperms in the lumen, testosterone producing leydig cells, sertoli cells enclosed in round, thick and fibrous capsule called tunica albuginea.

Aqueous *Aloe vera* gel extract treated animals (250 and 100 mg/ kg b.w.) [Figure 5.8 (c), (d)], [Figure 5.8 (e), (f)] attenuated the normal histological features resulting in wide spectrum of damage in testes like shrunken tubules, disorganized and distorted seminiferous tubules, depletion in cell population, abnormal widening of interstitial spaces was also observed. Aqueous *Aloe vera* gel extract treated animals (50 mg/ kg b.w.) revealed normal shape of seminiferous tubules [Figure 5.8 (g), (h)].

4.3. Effect of Various Doses of Aqueous *Aloe vera* Gel Extract on LDH Activities in Serum and Various Organs

A significant increase was observed in serum LDH activity upon administration of *Aloe vera* (250 mg/ kg body weight and 100 mg/ kg b.w.) animals when compared to control group. On the other hand, at the concentration of 50 mg/

kg b.w. LDH activities remained unaltered when compared with control group in serum, liver, spleen, kidney and testes [Figure 5.2], [Tables 5.5, 5.7, 5.9, 5.11].

4.4. Effect of Various Doses of Aqueous *Aloe vera* Gel Extract on LPO Level in Various Organs

Administration of *Aloe vera* (250 mg/ kg b.w.) showed significantly increased level of LPO in liver, spleen, kidneys, testes and blood as compared to control group, while at the concentration of (100 mg/ kg b.w.) resulted significant increase in LPO levels in kidneys and blood, when compared with the control group. *Aloe vera* (50 mg/ kg b.w.) did not alter the LPO levels in all the tissues when compared with the control group [Figure 5.3], [Table 5.6, 5.8, 5.10, 5.12].

4.5. Standardization of X-ray Dose

For the standardization of X-ray dose animals were randomly divided into two groups i.e. control and X-ray irradiated group. Animals were subjected to radiation exposures (0.258Gy twice a day for two days, 0.258 Gy twice a day for four days). 2 folds increase in LPO levels and LDH activity was observed in serum and blood of irradiated animals after two days of X-ray exposure when compared to control animals [Figures 5.9, 5.10]. These animals were then further exposed at the dose of 0.258Gy (twice a day) for four days in a week. This dose was selected for further studies based on significant increase (3 folds) in LDH activity and LPO levels in liver, spleen, kidney and testes when compared to four days of X-ray exposure [Tables 5.14, 5.15, 5.16, 5.17, 5.18,

5.19, 5.20, 5.21]. Histopathology, biochemical and various parameters were done after four days of X-ray exposure in plasma and different organ of interest.

4.6. Effect of Two Days and Four Days of X-ray Exposure on Body Weight, Diet Intake and Water Consumption

A significant decline in the body weight was observed in X-ray on last week (i.e. 4th week) of the two days and four days of exposures in mice when compared to their initial body weight on 1st week of the study [Table 5.13]. X-ray irradiated group exhibited a significant decrease in the diet intake on last week (i.e. 4th week) of irradiation when compared to the animals of control animals. Water consumption remained unaltered in all the treatment groups.

4.6.1. Histopathology of Liver

Histopathological analysis of Liver from control animals revealed normal histoarchitecture as mentioned above [Figure 5.11 (a), (b)] Histological alterations were detected in X-ray irradiated animals [Figure 5.11 (c), (d)] showing widening and dilated central vein with ruptured endothelial lining cells with congested plates of vascular sinusoidal spaces along with vascular channels radiating out from central veins when compared to control group [Figure 5.11(a), (b)].

4.6.2. Histopathology of Spleen

Histopathological analysis of spleen from control animals revealed normal architecture [Figure 5.12 (a), (b)]. Spleen of X-ray irradiated animals in histology [Figure 5.12 (c), (d)] showed decrease in the amount of white pulp

and increased red pulp. Large number of macrophages and reduction in a number of lymphocytes in red pulp was observed. Lymphocytes appeared to be more susceptible due to radiation injury. Infiltration in white pulp and thickened trabeculae was also observed as compared to control animals [Figure 5.12 (a), (b)].

4.6.3. Histopathology of Kidney

Histopathological examination of kidney from normal animals revealed normal histoarchitecture [Figure 5.13 (a), (b)]. Histoarchitecture of kidney section from X-ray irradiated animals [Figure 5.13 (c), (d)] resulted in decrease in number of Bowman's capsule, shrinkage of Bowman's capsule, glomerular attenuation and glomerular congestion. The thick and thin parts of loop of Henle and greater part of collecting ducts present in inner medulla revealed normal histoarchitecture when compared to control group [Figure 5.13 (a), (b)].

4.6.4. Histopathology of Testes

Histopathological analysis of testes from control animals revealed normal architecture [Figure 5.14 (a), (b)]. Testes section of X-ray irradiated animal [Figure 5.14 (c), (d)] revealed various pathological changes such as shrunken tubules, disorganized/distorted seminiferous tubules, depletion in germinal cell population, disrupted basement membrane, empty tubules, disordered and shrunken seminiferous tubules. Lumen is full of cellular and spermatogenic debris and thinning of seminiferous epithelium with loosely arranged cells were also observed as compared to control animal [Figures 5.14 (a), (b)].

4.7. Effect of X-ray/ or Aqueous *Aloe vera* gel Extract on Body Weight

Mice from all the groups were carefully examined for alterations in body weight, diet intake and water consumption throughout the experimental period. The animals exposed to radiation exhibited some signs of radiation sickness within two days. The main symptoms included decreased diet intake, irritability, hair ruffling and behavioural alterations. Body weight of control and *Aloe vera* treated animals remained unaltered throughout the experiment period and showed normal trend in maintaining body weight. However, a continuous decline in the body weight was observed in X-ray on last week (i.e. 4th week) of the exposure in mice when compared to their initial body weight on 1st week of the study. Significant decrease in body weight after X-ray irradiation in mice was observed when compared to the animals of control and *Aloe vera* treated group. Also, the body weight of mice in *Aloe vera* pretreated and X-ray irradiated group increased significantly when compared to X-ray irradiated group on last week of the exposure [Figure 5.15].

4.7.1. Effect of X-ray/ or Aqueous *Aloe vera* Gel Extract on Diet Intake and Water Consumption

Control and *Aloe vera* treated animals did not depict any alterations in the diet intake during the entire course of treatment. Animals of X-ray irradiated group exhibited a significant decline in the diet intake on last week (i.e. 4th week) of the X-ray exposure when compared to initial week of control and *Aloe vera* treated group. Likewise, *Aloe vera* administration to X-ray irradiated mice also

caused a significant increase in diet intake when compared to 1st week of the study. But the diet intake was comparatively more of *Aloe vera* pretreated and X-ray irradiated group when compared to the mice exposed to X-ray irradiation [Table 5.22]. Water consumption remained unaltered in all the treatment groups until the end of the treatment period [Table 5.23].

4.8. Effect of X-ray/ or Aqueous *Aloe vera* Gel Extract on Histopathology of Liver, Spleen, Kidney and Testes

To see the effect of aqueous extract of *Aloe vera* against X-ray induced alterations in various organs of mice. For this, male mice were randomly divided into four groups: control, *Aloe vera*, X-ray irradiated and *Aloe vera* pretreated and irradiated animals. After completion of respective treatments, animals were sacrificed after 24 hours, of the last day of X-Ray irradiation. Various tissues were excised and analysed for histoarchitectural alterations.

Histopathological analysis of liver from control animals revealed normal histoarchitecture as mentioned above [Figure 5.16 (a)]. *Aloe vera* extract (50 mg/ kg b.w.) treated animals [Figure 5.16 (b)] revealed normal architecture. Histological alterations were detected in liver of X-ray irradiated animals [Figure 5.16 (c)] showing widening and dilated central vein with ruptured endothelial lining cells with congested plates of vascular sinusoidal spaces along with vascular channels radiating out from central veins as compared to control group animals. X-ray irradiated animals pretreated with aqueous *Aloe vera* gel extract administration [Figure 5.16 (d)] revealed normal histoarchitecture of liver [Figure 5.16 (a)]

Histopathological analysis of spleen from control animals revealed normal architecture [Figure 5.26 (a)] which included normal splenic architecture consisting of areas of white pulp with germinal center and surrounding lighter marginal zone, and red pulp. White pulp contains lymphoid aggregations, mostly lymphocytes and macrophages which are arranged around the arteries. Red pulp has lots of vascular sinuses. There are connective tissues termed as trabeculae. *Aloe vera* extract treated animals [Figure 5.26 (b)] revealed normal architecture with normal areas of white pulp with germinal center and surrounding lighter marginal zone, and red pulp. Spleen of X-ray irradiated animals [Figure 5.26 (c)] showed decrease in the amount of white pulp and increased red pulp, large number of macrophages and reduction in a number of lymphocytes in red pulp was observed. Lymphocytes appeared to be more susceptible due to radiation injury. Infiltration in white pulp and thickened trabeculae was also observed as compared to control animals [Figure 5.26 (b)]. X-ray irradiated animals pretreated with *Aloe vera* administration [Figure 5.26 (d)] resulted in macrophages considerably less when compared to X-ray irradiated group. The decrease in white pulp as compared with the X-ray irradiated group [Figure 5.26 (c)] was also observed.

Histopathological examination of kidney from control animals revealed normal histoarchitecture [Figure 5.33 (a)]. A gross section of kidney corpuscle revealed two distinguished regions i.e. outer cortex and inner medulla. Outer cortex consist of a tuft of capillaries lined with epithelial cells, proximal and distal convoluted tubule (PCT and DCT) which are enclosed within a fibrous capsule called Bowman's capsule known as Renal corpuscle. The thick and

thin parts of loop of Henle and greater part of collecting ducts are present in inner medulla comprised of renal pyramids as explained. *Aloe vera* extract treated animals [Figure 5.33 (b)] resulted normal cortical labyrinth and medullary region. Histoarchitecture of kidney section from X-ray irradiated animals [Figure 5.33 (c)] resulted in decrease in number of Bowman's capsule, shrinkage of Bowman's capsule, glomerular attenuation and glomerular congestion. The thick and thin parts of loop of Henle and greater part of collecting ducts present in inner medulla revealed normal histoarchitecture. X-ray irradiated animals pretreated with *Aloe vera* administration [Figure 5.33(d)] resulted in normal histoarchitecture of cortex and medulla as observed in control group [Figure 5.34 (a)]. Medullary section of X-ray irradiated [Figure 5.34 (c)], *Aloe vera* alone group [Figure 5.34 (b)], *Aloe vera* pretreated irradiated group [Figure 5.34(d)] revealed normal histoarchitecture as in control group [Figure 5.34 (a)]

Histopathological analysis of testes from control animals revealed normal architecture [Figure 5.42 (a)] which included lumen of seminiferous tubules with normal spermatogonia which are in direct contact with epithelial basal lamina, diploid primary spermatocytes, spermatid with sperms in the lumen, testosterone producing leydig cells, sertoli cells enclosed in round, thick and fibrous capsule called tunica albuginea. *Aloe vera* extract treated animals [Figure 5.42 (b)] (50 mg/ kg b.w.) revealed normal architecture of tubules. Testes section of X-ray irradiated animal [Figure 5.42 (c)] revealed various pathological changes such as shrunken tubules, disorganized/ distorted seminiferous tubules, depletion in germinal cell population, disrupted basement

membrane, empty tubules, disordered and shrunken seminiferous tubules. Lumen was full of cellular and spermatogenic debris and thinning of seminiferous epithelium with loosely arranged cells were also observed as compared to control animal [Figure 5.42 (a)]. X-ray irradiated animals pretreated with *Aloe vera* administration [Figure 5.42 (d)] resulted in organized histoarchitecture of seminiferous tubules but revealed debris in lumen of testes section as compared to control and X-ray irradiated group [Figure 5.42 (a), (c)]

4.9. Effect of X-ray and/ or Aqueous *Aloe vera* Gel Extract on LDH Activities in Serum and Various Organs

A significant increase was observed in serum, liver, spleen and kidney LDH activities of X-ray irradiated group when compared with control ($p \leq 0.001$; $p \leq 0.001$) and *Aloe vera* treated groups ($p \leq 0.001$; $p \leq 0.01$). Administration of *Aloe vera* to X-ray mice caused a significant decrease in serum, liver, spleen, kidney and testes LDH activities when compared with control ($p \leq 0.001$; $p \leq 0.001$) group. LDH activity of X-ray irradiated *Aloe vera* pretreated group remained significantly decreased when compared with X-ray irradiated group ($p \leq 0.001$; $p \leq 0.01$) and significantly increased when compared with *Aloe vera* treated group ($p \leq 0.001$; $p \leq 0.01$) in kidney. Serum LDH activity of *Aloe vera* administered to X-ray irradiated group decreased when compared with X-ray irradiated group [Figure 5.17] [Tables 5.24, 5.28, 5.30].

4.10. Effect of X-ray and/ or Aqueous *Aloe vera* Gel Extract on Liver Function Test

The serum liver enzymes (SGPT, SGOT, Bilirubin, albumin/globulin ratio) were significantly elevated by X-ray irradiation when compared to control

group ($p \leq 0.001$; $p \leq 0.001$) and *Aloe vera* treated groups ($p \leq 0.001$; $p \leq 0.01$). *Aloe vera* administered to X-ray irradiated group showed significantly decreased activity when compared to X-ray irradiated group ($p \leq 0.001$; $p \leq 0.01$) [Figure 5.18]

4.11. Effect of X-ray and/ or Aqueous *Aloe vera* Gel Extract on Renal Injury Markers

A significant increase was observed in urea, creatinine and BUN levels in X-ray irradiated group when compared to control group ($p \leq 0.001$; $p \leq 0.001$) and *Aloe vera* treated groups ($p \leq 0.001$; $p \leq 0.01$). *Aloe vera* administered to X-ray irradiated group showed significantly decreased levels of urea, creatinine and BUN when compared to X-ray irradiated group ($p \leq 0.001$; $p \leq 0.01$) [Figure 5.35]. A significant decrease was observed in GFR of X-ray irradiated group when compared to control group ($p \leq 0.001$; $p \leq 0.001$) and *Aloe vera* treated groups ($p \leq 0.001$; $p \leq 0.01$). *Aloe vera* administered to X-ray irradiated group showed significantly increased GFR when compared to X-ray irradiated group ($p \leq 0.001$; $p \leq 0.01$) [Figures 5.35, 5.36]

4.12. Effect of X-ray and/or Aqueous *Aloe vera* Gel Extract on Sperm Motility, Sperm Count and Testosterone Levels

A significant decrease in percentage of sperm motility as well as sperm count was observed in X-ray irradiated group as compared to control ($p \leq 0.001$; $p \leq 0.001$) and *Aloe vera* treated group ($p \leq 0.001$; $p \leq 0.01$). A significant increase was also observed in the *Aloe vera* administered to X-ray irradiated groups when compared to X-ray irradiated group ($p \leq 0.001$; $p \leq 0.01$). Sperm count in *Aloe vera* treated group was significantly increased when compared to control group ($p \leq 0.001$; $p \leq 0.001$) [Figures 5.43, 5.44]

The levels of testosterone were significantly decreased in X-ray irradiated group as compared to control ($p \leq 0.001$; $p \leq 0.001$) and *Aloe vera* treated group ($p \leq 0.001$; $p \leq 0.01$). A significant increase was also observed in the *Aloe vera* pretreated to X-ray irradiated groups when compared to X-ray irradiated group ($p \leq 0.001$; $p \leq 0.01$). In *Aloe vera* treated group the levels of testosterone remained unaltered when compared to control group ($p \leq 0.001$; $p \leq 0.001$) [Figure 5.45]

4.13. Effect of X-ray and/ or Aqueous *Aloe vera* Gel Extract on Micronuclei Formation in Spleen

A significant increase was observed in percentage of micronucleus induction in splenic tissue of X-ray irradiated group when compared with control ($p \leq 0.001$; $p \leq 0.01$) and *Aloe vera* treated groups ($p \leq 0.001$; $p \leq 0.01$) [Figure 5.31]. Administration of *Aloe vera* to X-ray mice caused a significant decrease in micronucleus formation when compared with control ($p \leq 0.001$; $p \leq 0.01$) group. Micronucleus formation in *Aloe vera* pretreated and X-ray irradiated group remained significantly decreased when compared with X-ray irradiated group ($p \leq 0.001$; $p \leq 0.01$) and significantly increased when compared with *Aloe vera* treated group ($p \leq 0.001$; $p \leq 0.01$). Micronucleus formation was expressed as percentage number of micronucleated cells to cells having normal nuclei [Figure 5.32].

4.14. Effect of X-ray and/ or Aqueous *Aloe vera* Gel Extract on Chromosomal Aberration Assay in Liver

Control mouse showed a normal set of $2n=40$ chromosomes with minimum aberrations as shown in [Figure 5.25]. After X-ray exposure a significant

increase in the percentage incidence of aberrant cells in the liver was observed when compared with control ($p \leq 0.001$; $p \leq 0.01$) and *Aloe vera* treated group ($p \leq 0.001$; $p \leq 0.01$). X-ray irradiated group caused pulverization, breaks and multiple types of abnormal rearrangements in the chromosomes of X-ray irradiated groups and *Aloe vera* pretreated X-ray irradiated group. However, extensive pulverization was observed in X-ray irradiated group and mild to moderated pulverization was observed in *Aloe vera* pretreated X-ray irradiated group [Table 5.26]. Pretreatment with *Aloe vera* significantly decreased the X-ray induced increase in percentage incidence of aberrant cells and abnormalities when compared to X-ray irradiated group ($p \leq 0.001$; $p \leq 0.01$). No significant alterations were observed in the chromosomal aberration between control and *Aloe vera* treated group [Figure 5.24].

4.15. Effect of Aqueous *Aloe vera* Gel Extract on Cell Death Caused by X-ray Irradiation

4.15.1. TUNEL assay

Photomicrograph of TUNEL stained liver, spleen, kidney and testes showing blue stained nucleus in control and aqueous *Aloe vera* gel extract treated group. X-ray irradiated group showing positive apoptotic cells with brown stained nucleus when compared to control and X-ray exposed animals. X-ray irradiated group pretreated with aqueous *Aloe vera* gel extract revealed less number of apoptotic cells as compared to X-ray irradiated animals [Figures 5.19, 5.20, 5.37, 5.46]. Apoptotic index was found to be increased in X-ray irradiated animals when compared to control ($p \leq 0.001$; $p \leq 0.01$) and *Aloe vera* treated group ($p \leq 0.001$; $p \leq 0.01$) [Figures 5.20, 5.28, 5.38, 5.47]. However, a

significant decrease in percentage of apoptotic cells was observed in *Aloe vera* administered to X-ray irradiated animals when compared to X-ray irradiated group.

4.15.2. DNA fragmentation assay

DNA fragmentation assay performed in the liver, kidney and testes tissue of mice is shown in [Figures 5.21, 5.39, 5.48]. Agarose gel electrophoresis of genomic DNA extracted from each group was performed after X-ray exposure for the assessment of apoptosis. A single major and an intact DNA band was observed on gel indicating the integrity of genomic DNA. Apoptosis is associated with the cleavage of nuclear DNA into small sized fragments of low molecular weights. However, no significant fragments were observed in control and *Aloe vera* treated group. The result indicated that in X-ray irradiated group genomic DNA of X-ray irradiated group showed a diffused and mild smear indicating DNA damage when compared to control and *Aloe vera* extract treated group in different tissues. An intact genomic DNA band was observed in control and *Aloe vera* treated group.

4.16. Effect of X-ray/ or Aqueous *Aloe vera* Gel Extract on Antioxidant Defense System in Plasma and Various Tissues

4.16.1. GSH

Significant decrease in GSH activity in X-ray irradiated group was observed when compared with control ($p \leq 0.001$; $p \leq 0.001$) and *Aloe vera* treated group ($p \leq 0.001$; $p \leq 0.01$). *Aloe vera* treated irradiated animals showed a significant increase in GSH content when compared with X-ray irradiated group ($p \leq 0.001$; $p \leq 0.01$) in plasma, liver, spleen, kidney and testes. [Tables 5.25, 5.27, 5.29, 5.31, 5.32].

4.16.2. GR

A significant increase was observed in GR activity in X-ray irradiated group as compared to control ($p \leq 0.001$; $p \leq 0.001$) and *Aloe vera* treated group ($p \leq 0.001$; $p \leq 0.001$). *Aloe vera* pretreated and X-ray irradiated group showed significant decrease when compared to X-ray irradiated group ($p \leq 0.001$; $p \leq 0.001$). GR activity with administration of *Aloe vera* remained unaltered when compared with control in plasma and various organs ($p \leq 0.001$; $p \leq 0.001$) [Tables 5.25, 5.27, 5.29, 5.31, 5.32].

4.16.3. GSH-Px

The Gpx activity was observed to be significantly increased in X-ray irradiated group ($p \leq 0.001$; $p \leq 0.01$) when compared to the control group ($p \leq 0.001$; $p \leq 0.001$) and *Aloe vera* treated group ($p \leq 0.001$; $p \leq 0.001$) in plasma, liver, spleen, kidney and testes. Significant decrease was observed in *Aloe vera* pretreated X-ray irradiated group when compared with X-ray irradiated group ($p \leq 0.001$; $p \leq 0.01$) [Tables 5.25, 5.27, 5.29, 5.31, 5.32].

4.16.4. GST

GST activity increased significantly in plasma, liver, kidney and testes, while a significant decrease was observed in spleen when compared to the control ($p \leq 0.001$; $p \leq 0.001$) and *Aloe vera* treated group ($p \leq 0.001$; $p \leq 0.001$). However, when *Aloe vera* pretreated animals were challenged with X-ray irradiation, a significant decrease in GST activity was observed when compared to animals treated with X-ray only ($p \leq 0.001$; $p \leq 0.001$) except spleen [Tables 5.25, 5.27, 5.29, 5.31, 5.32].

4.16.5. CAT

The catalase activity was found to be increased significantly in X-ray irradiated group ($p \leq 0.001$; $p \leq 0.01$) when compared with the control group ($p \leq 0.001$; $p \leq 0.001$). Non-significant alterations were observed in the catalase activity of *Aloe vera* pretreated X-ray irradiated animals when compared to the control group ($p \leq 0.001$; $p \leq 0.001$). However, the activity was significantly decreased when compared to X-ray irradiated group ($p \leq 0.001$; $p \leq 0.01$) in plasma, liver, spleen, kidney and testes and significant increase was observed in *Aloe vera* treated group when compared with control group in testes only [Tables 5.25, 5.27, 5.29, 5.31, 5.32].

4.16.6. SOD

X-ray exposed group caused a significant decrease in spleen SOD activity and significantly increased in plasma, kidney and testes activity when compared with control ($p \leq 0.001$; $p \leq 0.001$) and *Aloe vera* treated group ($p \leq 0.001$; $p \leq 0.001$). Administration of *Aloe vera* to X-ray exposed group caused a significant increase in SOD activity in spleen, a significantly decreased activity was observed in plasma, kidney and testes when compared with X-ray exposed group ($p \leq 0.001$; $p \leq 0.001$). A significant decrease was observed in liver SOD activity in *Aloe vera* treated group, X-ray exposed group and *Aloe vera* pretreated X-ray irradiated group when compared with control group ($p \leq 0.001$; $p \leq 0.001$). In testes, *Aloe vera* pretreated X-ray exposed group activity remained unaltered when compared with control and *Aloe vera* treated group ($p \leq 0.001$; $p \leq 0.001$) [Tables 5.25, 5.27, 5.29, 5.31, 5.32].

4.17. Effect of X-ray/ or Aqueous *Aloe vera* Gel Extract on ROS and LPO in Blood and Various Organs

X-ray irradiation caused a significant increase in ROS and LPO levels in liver, spleen, kidney, testes and blood when compared with control ($p \leq 0.001$; $p \leq 0.001$) and *Aloe vera* treated groups ($p \leq 0.001$; $p \leq 0.01$). Significant decrease was observed in ROS and LPO level with administration of *Aloe vera* to X-ray irradiated mice when compared with X-ray irradiated group ($p \leq 0.001$; $p \leq 0.01$). No change was observed in the ROS and LPO level in *Aloe vera* treated group when compared with control group [Figures 5.22, 5.23], [Figures 5.29, 5.30], [Figures 5.40, 5.41], [Figures 5.49, 5.50], [Figures 5.52, 5.53].

4.18. Effect of X-ray and/ or Aqueous *Aloe vera* Gel Extract on Haematological Parameters

4.18.1. Differential Leukocyte Counts (DLC)

X-ray exposure led to a significant enhancement in neutrophil counts when compared to control ($p \leq 0.001$; $p \leq 0.01$) and *Aloe vera* treated mice ($p \leq 0.001$; $p \leq 0.01$). In contrast, pretreatment of *Aloe vera* to X-ray exposed mice induced a marked decrease in neutrophil counts in comparison to X-ray exposed group ($p \leq 0.001$; $p \leq 0.01$). No statistical alterations in the blood neutrophil counts were observed in *Aloe vera* and control group. Lymphocytes remain unaltered in X-ray irradiated, control, *Aloe vera* and *Aloe vera* administered to X-ray irradiated group [Table 5.33].

4.18.2. Red Blood Cell (RBC)

RBCs counts remained unaltered in both X-ray and *Aloe vera* pretreated X-ray exposed group when compared to control and *Aloe vera* treated mice [Table 5.33].

4.18.3. Haemoglobin (Hb)

Hb levels remained unaltered in both X-ray exposed and *Aloe vera* administration to X-ray exposed group when compared to control and *Aloe vera* treated mice [Table 5.33].

4.18.4. Total Leucocyte Counts (TLC)

Total leucocyte counts were found to be enhanced in X-ray irradiated group when compared to control ($p \leq 0.001$; $p \leq 0.01$) and *Aloe vera* treated group ($p \leq 0.001$; $p \leq 0.01$). However, in *Aloe vera* administered to X-ray irradiated group showed the elevation in TLC induced by X-ray was less in comparison to control mice ($p \leq 0.001$; $p \leq 0.01$). Moreover, no significant difference in the blood TLC was found in *Aloe vera* and control group [Table 5.33].

4.18.5. Platelets

Platelet counts were significantly elevated by X-ray irradiation when compared to control group ($p \leq 0.001$; $p \leq 0.01$) and *Aloe vera* treated groups ($p \leq 0.001$; $p \leq 0.01$). *Aloe vera* administered to X-ray irradiated group showed significantly decreased when compared to X-ray irradiated group ($p \leq 0.001$; $p \leq 0.01$) [Table 5.33].

4.19. Effect of X-ray and/ or Aqueous *Aloe vera* Gel Extract on Inflammatory Markers

Inflammatory markers (TNF- α , IL-6) were significantly elevated by X-ray irradiation when compared to control group ($p \leq 0.001$; $p \leq 0.01$) and *Aloe vera* treated groups ($p \leq 0.001$; $p \leq 0.01$). *Aloe vera* administered to X-ray irradiated group showed significantly decreased when compared to X-ray irradiated group ($p \leq 0.001$; $p \leq 0.01$) [Figure 5.54, 5.55].