Internationally indexed journal

Indexed in Chemical Abstract Services (USA), Index copernicus, Ulrichs Directory of Periodicals, Google scholar, CABI, DOAJ, PSOAP, EBSCO, Open J gate, Proquest, SCOPUS, EMBASE, etc.

Rapid and Easy Publishing

The “International Journal of Pharma and Bio Sciences” (IJPBS) is an international journal in English published quarterly. The aim of IJPBS is to publish peer reviewed research and review articles rapidly without delay in the developing field of pharmaceutical and biological sciences.

Indexed in Elsevier Bibliographic Database (Scopus and EMBASE)

SCImago Journal Rank 0.288
Impact factor 2.958*
Elsevier Bibliographic databases (Scopus & Embase)

**SNIP value** – 0.77

**SJR** - 0.288

**IPP** - 0.479

**SNIP** – Source normalised impact per paper

**SJR** – SCImago Journal rank

**IPP** – Impact per publication

*Source – www.journalmetrics.com (Powered by Scopus (ELSEVIER)*

---

And indexed/catalogued in many more universities

*Instruction to Authors visit [www.ijpbs.net](http://www.ijpbs.net)*

For any Queries, visit “contact” of www.ijpbs.net*
RADIATION AND CADMIUM INDUCED TESTICULAR INJURY AND ITS AMELIORATION BY ALOE VERA EXTRACT

PUROHIT RK*, BHATI SL, PURKHARAM, ISRARAN R, AGARWAL M AND CHAKRAWARTI A

Radiation Biology Laboratory, Department of Zoology, Govt. Dungar College, Bikaner (Rajasthan), India-334001 dr_rajendra_purohit@yahoo.co.in

ABSTRACT

Extract of Aloe vera leaves has been used for treatment of various diseases and disorders in ayurveda and unani medicine. Modulatory effect of Aloe vera against radiation and cadmium induced changes in terms of histological alterations in testis has been studied in present investigation. For this purpose mouse exposed to 7.0 Gy radiation showed radiation induced sickness, including marked changes in histology of testis. The Aloe vera leaf extract was given orally at a dose of 1000 mg/kg body wt/animal/day for five consecutive days prior to irradiation. The radiation dose reduction factor was 1.65. Aloe vera pre treatment significantly prevents radiation and cadmium induced histopathological changes in cells. The study suggested that Aloe vera extract has exerted significant radioprotective effects on testis. It is well known that Aloe is an essential component of antioxidant defense system with participation at multiple cellular levels. Aloe has a protective effect on histological damage by maintaining membrane integrity due to its direct action of free radicals. It can be concluded that Aloe has a protective role against cytotoxicity induced by radiation and cadmium in the testes of Swiss albino mice.

KEYWORDS: Aloe vera, Swiss albino mice, Radiation, Cadmium, Testes.

*Corresponding author
INTRODUCTION

Aloe vera has been appreciated as an important drug of the Indian system of medicine and used in ayurvedic preparation for the treatment of various ailments. It hepatoprotective, immuno modulatory and free radical scavenging properties. Deleterious effects of radiation on biological system have been shown in different ways therefore, various strategies have been developed to protect biological systems by means of chemical protection through interfering in the process of radiation damage. Aloe vera as an antioxidant has shown radioprotective effect on different mammalian organs against radiation. Cadmium is one of the most harmful heavy metal able to induce severe injury. Cadmium exerts its toxic effect via oxidative damage to cellular organelles by inducing the generation of reactive oxygen species. A oxidative stress is one of the important mechanism of cadmium induced damages, it can be expected that the administration of some antioxidant should be an important therapeutic approaches. The antioxidant properties of Aloe vera plays an important role in controlling oxidative stress. Consequently, this study has been performed to elucidate the possible radioprotective potential of the Aloe vera against radiation and cadmium induced testicular injury in Swiss albino mice. The irradiation can cause temporary azoospermia in mouse and this effect is reversible after 8 weeks.

MATERIALS AND METHODS

Maintenance of Animals
For the study, adult healthy male Swiss albino mice (6-8 weeks old) were procured from Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar (India). The animals were kept in polypropylene cages. They were fed with standard mice feed and water was given ad libitum. The cages were cleaned daily. The temperature of the room was maintained between 22 – 27°C. The Govt. Dungar College, Bikaner is registered under CPSCEA, Chennai, India (Registration no. 1066/ac/07/CPCSEA) and has its own Institutional Animal Ethics Committee (IAEC). The animals used for the present investigation were sacrificed strictly under the supervision of IAEC of the college.

Cadmium
Cadmium salt in the form of cadmium chloride (SDS Chemicals, India) was prepared by dissolving 20 mg of cadmium chloride in 1000 ml of the glass distilled water, thus giving a concentration of 20 ppm and then administered orally in drinking water.

Source and procedure of irradiation
Cobalt-60 gamma radiotherapy source (Theratron) of AECL make, obtained from Canada was used to expose the animals. This facility was provided by the Radiotherapy Department of Prince Bijay Singh Memorial Hospital, Bikaner (Rajasthan). Unanaesthetized mice were restrained in well ventilated boxes and exposed 7.0 Gy at the dose rate of 1.39 Gy/min.

Aloe vera extract
Fresh leaves of the Aloe vera were cleaned, cut into small pieces, air dried, powdered and extracted with double distilled water (DDW) by refluxing for 36 hrs. (12 hrs. x 3). The extract thus obtained was vacuum evaporated so as to make it in powder form. The extract was redissolved in DDW just before oral administration. An approximate thirty eight per cent yield of the extract was obtained. The drug was given from seven days prior to Cadmium chloride treatment or irradiation.

Survival assay
For survival studies mice of both control and experimental groups were exposed to
whole body gamma radiation and were checked daily for 30 days. The survival percentage of mice up to 30 days of exposure against each radiation dose was used to construct survival dose response curves. Regression analysis was done to obtain LD$_{50/30}$ values and to determine dose reduction factor (DRF).

**Plan of experimentation**

The animals were divided into the following groups

- **Group I** – Sham - irradiated animals (normal)
- **Group II** – Cadmium chloride treated animals
- **Group III** – Radiation treated animals (7.0)
- **Group IV** – Radiation and Cadmium chloride treated animals
- **Group V** – Cadmium chloride and Aloe vera treated animals
- **Group VI** – Radiation and Aloe vera treated animals
- **Group VII** – Radiation, Cadmium chloride and Aloe vera

**Autopsy schedule**

Animals from all the above treated groups (I to VII) were regularly observed till 30 day for their weight change, any sign of sickness morbility, fur and skin changes, behavioral toxicity, any visible abnormalities and mortality. All animals were necropsied at 1, 2, 4, 7, 14, and 28 day of post treatment for the evaluation at histological variation in testes.

**Histopathological analysis**

Testes were surgically removed at each autopsy interval from animals of each group and weighed, than fixed in bouin’s fixative, dehydrated and embedded in paraffin wax. 5 μm Slim serial sections were prepared from each testis. Slides were prepared by routine procedure and stained in haematoxyline and eosin. Histological alteration in the testicular architecture were observed in the seminiferous tubules. For the measurement of weight index, weight of both the testes from each animals was recorded.

**RESULTS**

Animals treated with Aloe vera showed slide / no significant changes in weight of the testis on day 1, 2, 4, 7, 14, and 28. Tubules showed all stages of normal germ cells with active spermatogenesis. Animals treated with radiation showed reduction in the testis weight during all day of observation whereas Aloe vera pretreated irradiated group, there was a significant increase in testis weight at all autopsy intervals as compared to irradiated animals. In irradiated mice, a drastic depletion of spermatogenic population with necrotic and pyknotic nuclei was observed. The germinal epithelium was highly disorganized with shrinkage of tubules and cytoplasmic vacuolation. Total absence of sperm and spermatids were observed. Sertoli cells and leydig cells showed shrinkage in their size. While mice pretreated with AVE exhibited, less severe damage in spermatogonia population and germinal epithelium. In irradiated mice, there was significant decrease in number of spermatogonia, primary spermatocyte, secondary spermatocyte and spermatid. Notably, in mice pretreated with AVE and then exposed to radiation dose, the quality ( as determined by intact germinal epithelium, no pyknosis, necrosis, karyolysis present, karyorrhexis less cytoplasmic vacuolization ) and number of germ cells increased by day 28. The histology of testis revealed near normal histoarchitecture except some cytoplasmic vacuolization and lumen with full sperms. The histological observation on the testes of the control group showed normal appearance of seminiferous tubules, spermatogenic cells and interstitial cells. The mice treated with cadmium showed many histological changes, oedema, degeneration of spermatogenic cells, pyknotic nucli and dilation and congestion of blood vessels. In addition, cadmium induced a pronounced alteration of the spermatogonic process with dramatical reduction of spermatozoa produced in the
lumen of the seminiferous tubules section. These histological changes were reduced in the Aloe vera treated experimental groups. Distorted architecture of seminiferous tubules was seen in the form of shrunken tubules, exfoliation, intertubular oedema, karyorrhexis, karyolysis, pycnotic nuclei, necrotic cells and with degranulated cytoplasm in irradiated mice. Intertubular edema and hemorrhage in intertubular space, distortion of cellular arrangement, shrinkage of tubules cellular vacuolization, lower rate of differentiating cells towards tubules centic and low mitotic index when compared with non irradiated mice.

Figure 1
Normal testicular architecture showing almost normal seminiferous tubules and spermatogonia.

Figure 2
Day-1 after cadmium chloride treatment showing slightly damaged tubular and cellular structure.
Figure 3
Day-4 after irradiation depicting cytoplasmic degranulation, vacuolation, necrosis and pycnotic nuclei. At certain places giant cells and leucocytic infiltration existed. Distortion of germinal epithelium is also evident.

Figure 4
Day-7 after combined treatment of radiation and cadmium chloride displaying karyolysis, karyorrhexis, intertubular oedema. Hyperaemia of blood vessels is also clearly visible, leucocytic infiltration is also evident.
Figure 5
Day-14 after administration or Aloe vera with combined treatment of radiation and cadmium chloride exhibiting comparatively better testicular architecture. The less severe oedema and better condition of spermatids and sperms is visible.

Figure 6
A bunch of sperms displaying normal conditions of head and tail. Many sperms are in coiling condition.
DISCUSSION

Infertility has been a major medical and social preoccupation. The interaction of radiation with component of living system induced cell death, may be result of accumulation of free radicals, LPO, disruption in the membrane including cellular, nuclear and organelle, leakage of lysosomal acid phosphatase, changes in the surface properties of chromosomal leading to stickiness, breakage of double strand of DNA and chromosomal aberration. The protective ability of the phyto chemicals against radiation and cadmium induced male reproductive abnormalities may offer a new insight into the modification of testicular germ cell radio sensitivity which may have implication in amelioration of testicular injuries. Therefore, the major concern of the present investigation is to assess the possible radioprotective capability of Aloe vera extract in clinical field against cadmium and radiation induced male reproductive dysfunction. It has been suggested by Ellis and Berliner, 1969 that 17 β-ol-dehydrogenase (oxidase) is located in both tubules and interstitial elements and diminishes progressively during the interval after irradiation. This phenomenon is directly related to the destructive changes associated with the depopulation of the seminiferous tubules. They also suggested the destruction of the germinal epithelium with direct or whole body irradiation, that affect both the reduction of androstenedione to testosterone and the oxidation of testosterone to androstenedione. Thus, a very dynamic relationship seems to exist between androgen synthesis and spermatogenesis. Closely associated with changes in androgen synthesis is the effect of radiation on DNA synthesis. Thus, it can be said that although Sertoli and Leydig cells are histologically insensitive they are "biochemically radiosensitive". There is no doubt that the significant increase in survival in Aloe Vera treated mice could be due to the antioxidant property, as evidenced by the restoration of glutathione and LPO levels. Gamma radiation exert its harmful effect through the generation of free radicals. These free radicals known as ROS could damage radiosensitive cells such as spermatogonia by disturbing normal metabolism, proliferation and differentiation which may lead to mutagenesis, apoptosis and necrosis. On the other hand Aloe vera might preserve cell proliferation by scavenging free radicals. This ability introduce Aloe vera as a potent radioprotective against cadmium and gamma radiation. On the other hand, an ideal radio protector should have the ability to scavenge free radicals and oxidative damage and facilitate DNA repair. All of these evidence indicate that Aloe may mitigate the harmful effect of radiation on spermatogonia by its radical scavenging and this finding is supported by remarkable increase in mean sperm count in mice. Supplementation of Aloe vera might have accountable for the increased concentration of phyto-antioxidants which seems to be a responsible aspect for lowering the lipid peroxidation is not only the free radicals but also the low levels of antioxidants that scavenge them. Alternatively, Aloe might have increased the intracellular levels of reduced glutathione and provides protection against cadmium and radiation induced mortality. Cadmium has been demonstrated to stimulate free radical production, resulting in oxidative deterioration of lipids, proteins, DNA and various pathological condition in humans and animals. Both types of cadmium exposure acute as well as chronic is associated with elevated lipid peroxidation in various tissue. The endothelial damage of small blood vessels, oedema and haemorrhage of the mice testis can be demonstrated by using just a single parental dose of Cadmium chloride at 2 - 4 mg/kg. Cadmium related changes in testicular histopathology have implicated testicular blood vessel damage, followed by the degeneration of spermatopoietic
epithelium as the main cause of cadmium toxicity\textsuperscript{13,14,15}. The changes observed were more severe in the testis of combined treatment animals (both radiation and cadmium chloride treated) as compared to individual one. The damage and recovery pattern showed a dose dependent and synergistic action\textsuperscript{16}. The \textit{Emblica officinalis} Garten fruits extract ameliorates reproductive injury and oxidative testicular toxicity induced by Chlorpyrifs in male rats. They concluded that aqueous extract of \textit{Emblica officinalis} formulation, an herbal preparation ameliorate male reproductive tissue damage\textsuperscript{17}. The radioprotective potential of \textit{Punica granatum} fruit rind extract in mouse testes after exposing the animals with 8 Gy of gamma radiation. They reported an increase in the value of total proteins and RNA contents and decrease in testes weight. They concluded that \textit{Punica granatum} fruit rind extract pretreatment renders protection against biochemical changes in mouse testes\textsuperscript{18}.

**CONCLUSION**

It can be concluded that \textit{Aloe vera} is potent enough to check radiation and cadmium induced histopathological and biochemical changes in the testes of Swiss albino mice. If certain herbal drugs like \textit{Aloe vera} given to the cancer patient during radiotherapy, they can minimize the side effects caused by the radiation.

**ACKNOWLEDGEMENT**

Authors gratefully acknowledge the Head, Department of Zoology and the Principal, Govt. Dungar College, Bikaner (India) for providing necessary facilities in the department. The irradiation facility provided by the Radiotherapy Department, Acharya Tulsi Cancer Hospital and Research Centre, PBM Hospital, Bikaner (India) is also gratefully acknowledged.

**REFERENCES**

8. Goel HC, Samanta N, Kannan K, Kumar PI and Bala M, Protection of...


