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Protective Effect of Picrorhiza kurroa Against Gamma Radiation and Mercuric Chloride-Induced Jejunal Injury in Swiss Albino Mice

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Abstract

Ionizing radiation is an important therapeutic modality in cancer treatment; however, exposure to radiation may produce significant injury to normal tissues, particularly the rapidly proliferating cells of the gastrointestinal tract. The small intestine is highly radiosensitive and may undergo severe structural and biochemical alterations following irradiation. Environmental and occupational exposure to heavy metals such as mercury may further aggravate tissue injury. The present study was undertaken to evaluate the protective efficacy of *Picrorhiza kurroa* against gamma radiation- and mercuric chloride-induced alterations in the jejunum of Swiss albino mice.

Healthy adult Swiss albino mice aged 6–8 weeks were divided into experimental groups receiving mercuric chloride, gamma radiation, combined radiation and mercuric chloride, and corresponding treatments with *Picrorhiza kurroa*. Mercuric chloride was administered at 0.5 ppm through drinking water, while animals were exposed to sublethal doses of 2.5 Gy or 5.0 Gy gamma radiation from a Cobalt-60 source. *Picrorhiza kurroa* extract was administered orally at 150 mg/kg body weight/day, beginning seven days before irradiation and/or mercuric chloride exposure and continuing until the final autopsy interval. Animals were examined at 1, 2, 4, 7, 14 and 28 days after treatment.

Histological parameters included villus length, goblet cell number, mitotic activity, abnormal mitoses, dead cells and total crypt cell population. Biochemical parameters included total proteins, cholesterol, acid phosphatase, alkaline phosphatase, DNA and RNA. Radiation and mercuric chloride produced marked histopathological and biochemical alterations in the jejunum. The combined treatment produced more severe changes than either treatment alone, indicating a synergistic detrimental effect. Radiation-induced changes were dose dependent, with greater severity at 5.0 Gy. Treatment with *Picrorhiza kurroa* considerably reduced the severity of tissue damage and promoted earlier recovery. The drug-treated groups demonstrated earlier restoration of villus length, goblet cell population, mitotic activity, crypt cell population, DNA, RNA, protein and cholesterol levels, together with comparatively rapid normalization of enzyme activities.

The findings indicate that *Picrorhiza kurroa* possesses significant protective potential against radiation- and mercuric chloride-induced jejunal injury. Its protective action appears to involve multiple biological mechanisms. *Picrorhiza kurroa* may therefore represent a promising natural agent for reducing radiation-associated intestinal injury.

Keywords: *Picrorhiza kurroa*; Kutki; gamma radiation; mercuric chloride; jejunum; radioprotection; enteroprotection; Swiss albino mice; histopathology; DNA; RNA.

1. Introduction

Radiation therapy has become an essential component of modern cancer treatment and has contributed substantially to cancer control and cure. Nevertheless, irradiation may also damage normal tissues surrounding or included within the treatment field. Among the important complications of radiation exposure is injury to the gastrointestinal tract, particularly the small intestine. The intestinal epithelium contains rapidly proliferating cells and is consequently highly sensitive to ionizing radiation. Radiation-induced injury may involve cellular, vascular and connective-tissue components and may result in both acute and delayed pathological changes.

The increasing use of ionizing radiation in clinical, industrial and other applications has made radiation protection an important area of biological research. In addition to therapeutic exposure, organisms may encounter radiation through occupational, environmental and accidental sources. The biological consequences of radiation exposure are therefore of continuing scientific and public-health interest.

Considerable research has been undertaken to identify agents capable of protecting normal tissues from radiation-induced damage. Although several synthetic chemical compounds have demonstrated radioprotective activity, their practical application may be restricted by toxicity and other adverse effects at effective concentrations. Medicinal plants and their bioactive preparations have consequently received increasing attention as potential sources of comparatively safer radioprotective compounds.

Picrorhiza kurroa is a medicinal plant traditionally used for various therapeutic purposes. The present investigation was designed to evaluate its protective efficacy against radiation and heavy-metal-induced injury in the jejunum. In particular, the study examined whether pretreatment with *Picrorhiza kurroa* could reduce the qualitative and quantitative histological changes and biochemical alterations produced by gamma radiation and mercuric chloride, individually and in combination.

Aim of the Study

The objective of the present investigation was to evaluate the protective efficacy of *Picrorhiza kurroa* against gamma radiation- and mercuric chloride-induced quantitative and qualitative alterations in the jejunum of Swiss albino mice.

2. Materials and Methods

2.1 Experimental Animals

Healthy adult Swiss albino mice aged 6–8 weeks were used for the investigation. The animals were randomly allocated to experimental and control groups. The experimental design included sham-irradiated controls, mercuric chloride-treated animals, irradiated animals, animals receiving combined radiation and mercuric chloride, and corresponding groups treated with *Picrorhiza kurroa*.

2.2 Experimental Groups

Group I – Sham-irradiated control

Animals were sham-irradiated and served as the normal control group.

Group II – Mercuric chloride

Animals received mercuric chloride at a concentration of 0.5 ppm *ad libitum* through drinking water throughout the experimental period.

Group III – Gamma radiation

Animals were exposed to sublethal doses of gamma radiation and subdivided into:

- Group IIIa: 2.5 Gy
- Group IIIb: 5.0 Gy

Group IV – Gamma radiation + mercuric chloride

Animals received mercuric chloride at 0.5 ppm and gamma radiation:

- Group IVa: 2.5 Gy + mercuric chloride
- Group IVb: 5.0 Gy + mercuric chloride

The combined-treatment groups were designed to evaluate the interaction between radiation and mercury-induced toxicity.

Group V – Mercuric chloride + *Picrorhiza kurroa*

Animals received mercuric chloride at 0.5 ppm and *Picrorhiza kurroa* extract orally at 150 mg/kg body weight/day. Treatment with *Picrorhiza kurroa* was initiated seven days before mercuric chloride exposure and continued until the final autopsy interval.

Group VI – Gamma radiation + *Picrorhiza kurroa*

Animals received *Picrorhiza kurroa* for seven days before irradiation and throughout the experimental period:

- Group VIa: 2.5 Gy + *Picrorhiza kurroa*
- Group VIb: 5.0 Gy + *Picrorhiza kurroa*

Group VII – Gamma radiation + mercuric chloride + *Picrorhiza kurroa*

Animals received mercuric chloride and *Picrorhiza kurroa* at 150 mg/kg body weight/day. *Picrorhiza kurroa* treatment commenced seven days before irradiation and mercuric chloride exposure.

- Group VIIa: 2.5 Gy + mercuric chloride + *Picrorhiza kurroa*
- Group VIIb: 5.0 Gy + mercuric chloride + *Picrorhiza kurroa*

2.3 Autopsy Schedule

A minimum of five animals from Groups II–VII were sacrificed at 1, 2, 4, 7, 14 and 28 days after treatment. Body weight was recorded before autopsy. Five animals from the sham-irradiated control group were similarly examined.

2.4 Histological Studies

The jejunum was examined for qualitative and quantitative changes involving the crypts and villi.

Qualitative parameters

Histological alterations in the different layers of the intestine, including the crypts and villi, were assessed.

Quantitative parameters

The following parameters were evaluated:

1. Villus length
2. Goblet cells per crypt section
3. Goblet cells per villus section
4. Mitotic figures per crypt section
5. Abnormal mitotic figures per crypt section
6. Dead cells per crypt section
7. Total cells per crypt section

These parameters were selected to assess epithelial integrity, cellular proliferation, cell death and intestinal recovery.

2.5 Biochemical Studies

The following biochemical parameters were assessed in the jejunal tissue:

- Total protein – Lowry et al. (1951)

- Cholesterol – Oser (1965)
- Acid phosphatase – Fiske and Subbarow (1925)
- Alkaline phosphatase – Fiske and Subbarow (1925)
- DNA – Ceriotti (1952)
- RNA – Ceriotti (1955)

The biochemical investigations were used to complement the histological assessment of radiation- and mercury-induced injury.

3. Results

3.1 Histopathological Alterations

Mercuric chloride treatment produced distinct pathological changes in the jejunum, including loosening of the submucosa, hydropic degeneration, abnormal mitotic figures, pyknotic nuclei, cytoplasmic degranulation and shortening and loosening of the villi. Leucocytic infiltration was also observed in the lamina propria. In the non-treated groups, recovery became evident from approximately day 14, whereas *Picrorhiza kurroa*-treated animals showed signs of recovery as early as day 7.

Gamma irradiation produced dose-dependent histopathological alterations. These included loosening of musculature, hydropic degeneration of the submucosa and lamina propria, hyperaemia, haemorrhage, pyknotic cells, cytoplasmic degranulation, vacuolation and abnormal mitotic figures. At 5.0 Gy, more severe nuclear alterations such as karyolysis, karyorrhexis and chromatolysis were also observed. Villus shortening and fragmentation, leucocytic infiltration and accumulation of cellular debris were additionally recorded.

Combined exposure to radiation and mercuric chloride produced lesions of the same general nature as the individual treatments but with greater severity, demonstrating the detrimental interaction of the two stressors.

In contrast, animals treated with *Picrorhiza kurroa* showed considerably less severe pathological alterations and an earlier and faster recovery.

3.2 Villus Length

Villus length was reduced up to day 14 in animals receiving mercuric chloride, radiation or combined treatment. In *Picrorhiza kurroa*-treated animals, reduction was limited to day 7, followed by progressive recovery from day 14 through day 28. The reduction in villus length was more pronounced following combined exposure, whereas pretreatment with *Picrorhiza kurroa* substantially mitigated villus shortening.

The findings are consistent with impaired crypt-cell proliferation following radiation and mercuric chloride exposure, since epithelial cells generated in the crypts migrate toward the villi. Earlier recovery in the *Picrorhiza*-treated groups indicates preservation or restoration of proliferative activity.

3.3 Goblet Cells

Goblet cells per crypt section decreased markedly during the early post-treatment period. In the non-drug-treated groups, the reduction persisted through day 14, whereas in the *Picrorhiza*-treated groups it was restricted largely to the first seven days, followed by recovery.

Similarly, goblet cells per villus section decreased up to day 14 in untreated experimental groups, while the decline in *Picrorhiza*-treated animals was limited to day 7. Combined exposure produced more pronounced alterations than individual treatments, whereas *Picrorhiza kurroa* pretreatment reduced the loss of goblet cells.

3.4 Mitotic Activity

Mitotic figures decreased sharply after treatment in all experimental groups. The reduction persisted up to day 14 in non-drug-treated animals but only up to day 7 in *Picrorhiza*-treated groups, followed by progressive restoration from day 14 to day 28. The decline was greatest following combined radiation and mercuric chloride exposure.

3.5 Abnormal Mitoses

Abnormal mitotic figures increased during the early post-treatment period. The increase was more persistent in the non-drug-treated groups. The severity of mitotic abnormalities was dose dependent, and an inverse relationship was observed between abnormal and normal mitotic figures. The findings indicate that mitotic abnormalities interfered with effective crypt-cell proliferation.

3.6 Cell Death and Total Crypt Cell Population

Dead cells increased substantially following radiation and/or mercuric chloride exposure. In non-drug-treated animals, the increase continued up to day 7, whereas *Picrorhiza*-treated groups showed an increase only up to day 4, followed by a marked decline from day 7 onward.

Total crypt cell numbers decreased up to day 14 in non-drug-treated animals and recovered by day 28. In *Picrorhiza*-treated animals, the decrease was restricted to approximately day 7, followed by progressive recovery. The maximum reduction in crypt cell population coincided with minimal mitotic activity and increased cell death.

4. Biochemical Results

4.1 Total Protein

Total protein content decreased following radiation and/or mercuric chloride exposure. The decrease was more pronounced in non-drug-treated animals, whereas *Picrorhiza kurroa* treatment reduced the magnitude of the decline and promoted earlier recovery. Combined radiation and mercuric chloride exposure produced a particularly pronounced reduction, which was attenuated by *Picrorhiza*.

4.2 Cholesterol

Jejunal cholesterol levels decreased from day 1 and remained reduced up to day 14 in the non-drug-treated groups. Recovery was incomplete by day 28. In contrast, *Picrorhiza*-treated groups showed a shorter period of decline and earlier recovery. The reduction was dose dependent, and *Picrorhiza* treatment resulted in comparatively less severe alterations.

4.3 Acid Phosphatase

Acid phosphatase activity increased significantly in non-drug-treated groups from day 1 through day 14 ($P < 0.001$), followed by a decline on day 28. In *Picrorhiza*-treated animals, the increase persisted only up to day 7, followed by a significant decline from day 14 onward ($P < 0.001$).

4.4 Alkaline Phosphatase

Alkaline phosphatase activity increased through day 14 in non-drug-treated groups and subsequently declined. In *Picrorhiza*-treated animals, the increase was limited to approximately day 7, followed by gradual reduction through day 28. The less pronounced increase in enzyme activity in treated groups indicates an ameliorative effect of *Picrorhiza kurroa*.

4.5 DNA

DNA content decreased in all experimental groups in a dose-dependent manner. Non-drug-treated groups showed a decline up to day 14, whereas *Picrorhiza*-treated groups showed a shorter period of decline, followed by recovery from day 14 onward. The comparatively smaller reduction and earlier

restoration of DNA content suggest protection against radiation- and mercuric chloride-induced cellular damage.

4.6 RNA

RNA concentration decreased in all experimental groups. The reduction persisted through day 14 in non-drug-treated animals, with only partial recovery by day 28. In *Picrorhiza*-treated animals, RNA decreased up to day 7 and subsequently increased from day 14 onward. The less severe reduction in RNA further supports the protective effect of *Picrorhiza kurroa*.

5. Discussion

The present investigation demonstrates that both gamma radiation and mercuric chloride produce substantial structural and biochemical injury in the jejunum of Swiss albino mice. The gastrointestinal epithelium is particularly vulnerable because maintenance of intestinal architecture depends upon continuous proliferation and differentiation of crypt epithelial cells.

The observed reduction in villus length, goblet cell number and mitotic activity following radiation and mercury exposure indicates impairment of intestinal epithelial renewal. The increase in abnormal mitoses and dead cells further demonstrates disruption of normal crypt-cell proliferation. The close association between decreased mitotic activity, reduced crypt cell population and increased cell death suggests that impaired cellular proliferation is an important component of the observed intestinal injury.

Radiation-induced changes were dose dependent. The 5.0 Gy exposures produced more severe nuclear and cytoplasmic abnormalities than the lower radiation dose. This dose-response pattern was also reflected in the quantitative parameters and biochemical alterations.

An important finding of the present study was the greater severity of injury following combined exposure to gamma radiation and mercuric chloride. The combined treatment produced more pronounced histological and biochemical alterations than either treatment alone. This supports a synergistic detrimental interaction between the two environmental/experimental stressors.

Pretreatment with *Picrorhiza kurroa* substantially modified these adverse effects. In treated animals, the duration of reduction in villus length, goblet cell population and mitotic activity was shorter, while recovery occurred earlier. Similarly, the increase in dead cells and abnormal mitoses was comparatively less severe. These observations indicate that *Picrorhiza kurroa* helps preserve intestinal epithelial integrity and facilitates regeneration following injury.

The biochemical observations provide additional evidence for this protective effect. Protein, cholesterol, DNA and RNA contents were less severely reduced in *Picrorhiza*-treated animals and recovered earlier. Similarly, the radiation- and mercury-associated alterations in acid and alkaline phosphatase activities were comparatively less pronounced in treated animals.

The mechanism responsible for the observed protection cannot be established definitively from the present data alone. However, the findings suggest that the protective action of *Picrorhiza kurroa* is multifactorial and may involve preservation of cellular integrity, maintenance of proliferative activity and facilitation of tissue recovery. The source data specifically indicate that its radioprotective effect appears to involve multiple mechanisms associated with the multiplicity of its biological properties.

6. Conclusion

The present study demonstrates that gamma radiation and mercuric chloride induce significant histological and biochemical alterations in the jejunum of Swiss albino mice. The severity of radiation-

induced damage was dose dependent, and combined exposure to radiation and mercuric chloride produced more pronounced injury than either treatment individually.

Pretreatment with *Picrorhiza kurroa* at 150 mg/kg body weight/day reduced the severity of radiation- and mercuric chloride-induced lesions and accelerated recovery. The protective effect was evident in both histological and biochemical parameters, including villus length, goblet cell population, mitotic activity, abnormal mitoses, cell death, crypt cell population, protein, cholesterol, phosphatase activities, DNA and RNA.

Overall, *Picrorhiza kurroa* demonstrated promising enteroprotective/radioprotective potential in this experimental model and may warrant further investigation as a natural agent for reducing radiation-associated intestinal injury. The source study similarly concludes that *Picrorhiza* may have potential for reducing radiation-induced side effects in patients undergoing radiotherapy.

7. Key Findings

1. Gamma radiation caused dose-dependent jejunal injury.
2. Mercuric chloride produced significant histological and biochemical alterations.
3. Combined radiation and mercuric chloride exposure produced synergistic detrimental effects.
4. Radiation and mercury reduced villus length, goblet cells, mitotic activity and total crypt cell population.
5. Abnormal mitoses and dead cells increased following treatment.
6. Protein, cholesterol, DNA and RNA contents were reduced after exposure.
7. Acid phosphatase and alkaline phosphatase activities were altered following exposure.
8. *Picrorhiza kurroa* reduced the severity of these changes.
9. *Picrorhiza*-treated animals showed earlier and faster recovery.
10. The protective effect was observed at both radiation dose levels, with and without mercuric chloride exposure.

8. References

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