ALTERATIONS IN PHOSPHATASES ACTIVITY IN BLOOD OF MICE BY ALSTONIA SCHOLARIS EXTRACT AFTER GAMMA IRRADIATION

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Summary

This study intended to investigate the radio-protective effects of *Alstonia scholaris* bark extract (ASE) on biochemical constituents in peripheral blood. For this purpose, Swiss albino mice were selected from an inbred colony and assorted into 4 groups. Animals in **Group I** were administered with double distilled water (DDW), volume equal to ASE, by oral gavage to serve as vehicle treated control. Mice in **Group II** were administered orally ASE once daily at a dose of 100 mg/kg b. wt. / animal/day for 5 consecutive days. In **Group III,** animals were given DDW for 5 consecutive days then exposed to 7.5 Gy gamma radiation. **Group IV** mice were treated with ASE (as in Group-II), and were exposed to 7.5 Gy after half an hour of the last administration of ASE. Animals of each group were autopsied on 12 hrs, days 1, 3, 7, 15 & 30 post-treatment intervals for biochemical estimation in blood. The values of serum acid phosphatase were significantly higher in irradiated control as compared to vehicle treated; whereas, animals pretreated with ASE showed a significant decline in these values at all the autopsy intervals. A significant decrease below the normal in alkaline phosphatase activity was measured after irradiation but the same was found to be significantly higher throughout the experiment in ASE pretreated group.

Key words: Radiation protection, Serum phosphatases , *Alstonia scholaris,* Swiss albino mice

Introduction

Life has developed under the continuous exposure to telluric and comic radiations. In the last century, the applications of nuclear resources for energy production, medical purposes or military uses has considerably increased at the doses to which man might be exposed. Radiation is the most studied environmental hazard in the world. Ionizing radiations including alpha, beta and gamma rays and neutrons with sufficient energy to generate ion pairs i.e. electrons, which can generate chemically active free radicals that in turn can damage the molecular structure resulting in to cellular or genetic dysfunctions [1] .

Advancement in use of radiation-based technologies in general will result in an increased radioactive waste which necessitates an improved control for the storage and transport. Clearly, it will need to have a strong voice in preparing the radiation protection standards that will ultimately have an impact on development at the global scale. The achievements to strengthen radiation protection over the past few years are truly remarkable.

Earlier the chemical substances, which could minimize the pathological change in the living systems after exposure to ionizing radiation, were looked into. Therefore, attempts were made in this direction from early 60's but the agent discovered so far are not safe to use for human beings due to high toxicity at their effective dose level. Some of them are cysteine [2], WR-2721[3], deoxyspergulin[4] , lipoic acid [5], diltiazem [6] and melatonin [7].

Recently, focus of radiation protection has shifted to test the radio-protective potential of plants and herbs in the hope that one day it will be possible to find a suitable pharmacological agent(s) that could protect humans against the deleterious effects of ionizing radiation in clinical and other conditions as well as during nuclear war or terrorist attack. These herbal drugs offer an alternative to the synthetic compounds and have been considered either non-toxic or less toxic, and this has given impetus to screen for their radio-protective ability.

Studies carried out during the past decade and a half have shown that the many herbal preparations like *Panax ginseng* [8] , *Emblica officinalis* [9], *Rosmarinus officinalis* [10] *Alstonia scholaris* [11], *Trigonella foenum graecum*[12] and *Tribulus terrestris* [13] protected mice against the radiation-induced sickness, mortality, dermatitis, spleen injury, liver damage, decrease in the peripheral blood cells, lipid peroxidation and cytogenetic lesions.

Alstonia scholaris, a tree belonging to family Apocynaceae, is a popular remedy in India for the treatment of various types of disorders in both the Ayurvedic and folkfore systems of medicine [14]. It grows throughout India in deciduous and ever green forests and also in plains. In folk medicine, its milky juice is applied on wounds, ulcers and rheumatic pains; mixed with oil and dropped into ear, it relieves ear ache. Juice of the leaves and tincture of the bark act in certain cases as a powerful galactogogue. The plant is reported to have anti-mutagenic effect Lim *et al.*[15]. The methanolic extract of this plant was found to exhibit pronounced anti-plasmodial activity [16].

Radiological accidents among groups of individuals often require a rapid evaluation of the absorbed dose in order to identify those individuals who must receive an adequate therapy. Physical, clinical and biological dosimetry are usually combined for the best dose assessment. Because of the practical limits of physical and clinical dosimetry, many attempts have been made to develop a biological dosimetry based upon changes in biological parameters. Interest of biochemistry in radiobiology was initially based on accessibility of techniques, rapidity of dose assessment and specific response for heterogenous irradiation that had proved to be a valuable parameters for diagnostic and prognostic purposes in many diseases.

Therefore, the present communication deals with the radiomodulatory effects of *Alstonia scholaris* extract in mice by taking some biochemical end points.

Materials and Methods

Animal care and handling

Male Swiss albino mice (*Mus musculus*), 6-8 weeks old weighing 20-24 g., from an inbred colony were used for the present study. The animals were provided standard mice feed (procured from Ashirwad Industries Chandigarh, India) and water *ad libitum*, and were maintained under controlled conditions of temperature and light (Light: dark, 10 hrs: 14 hrs.). Four animals were housed in a polypropylene cage with locally procured paddy husk (*Oryza sativa*) as bedding throughout the experiment. Tetracycline-containing water (0.13 mg/ml) was provided once a fortnight as a preventive measure against microbial infections. Animal care and handling were performed according to the guidelines set by the World Health Organization (WHO), Geneva, Switzerland and the INSA (Indian National Science Academy), New Delhi, India. The Departmental Animal Ethical Committee approved the present study.

Irradiation

Cobalt teletherapy unit (ATC-C9) at the Cancer Treatment Centre, Radiotherapy Department, SMS Medical College & Hospital, Jaipur was used for the irradiation. Unanaesthetised animals were restrained in well-ventilated perspex boxes and exposed to gamma radiation at the source to surface distance (SSD) of 77.5 cm to deliver the dose-rate of 1.32 Gy/min.

Plant material & extract preparation

The bark of *Alstonia scholaris* (Sapthaparna) was collected after proper identification (Voucher No. RUBL-19939) by a competent botanist in herbarium of Department of Botany, University of Rajasthan. Jaipur (India). The plant bark was powdered in a mixture and the extract was prepared by refluxing with the double distilled water (DDW) for 36 hrs (12 × 3) at 40^0 C. The liquid extract was cooled and concentrated by evaporating its liquid contents in an incubator. The extract was stored at the low temperature until its further use. The required dose for the treatment was prepared by dissolving the drug pellets in double distilled water and administered by oral gavage with a micropipette (100 µl/ animal) at a dose of 100 mg/ kg b. wt./animal. Henceforth, in this article bark extract of *Alstonia scholaris* will be termed as ASE

Evaluation of radiomodulatory influence of ASE

Experimental design

A total of 70 animals used for the experiment were assorted into 4 groups. Mice of Group 1 (Vehicle treated control, n=5) were orally administered double distilled water (DDW), volume equal to ASE. Animals belonging to Group II (ASE treated, n=5) were given Alstonia extract daily at a dose of 100 mg/ kg/ animal for 5 consecutive day. Animals of Group III (irradiated control, n=30) were exposed to 7.5 Gy gamma rays alone 30 min. after DDW administration on day 5^{th}. Group IV (ASE experimental, n=30) received ASE (100 mg/ kg b. wt. / animal) as in Group II. Half an hr after the last administration of ASE, such mice were exposed to above used radiation dose. A minimum of 5 animals

from group III & IV were necropsied on 12 hrs. , day 1, 3, 7, 15 and 30 post-treatment and their blood was taken from orbital sinus to measure serum acid and alkaline phosphatase activity by Kind and King's method using commercially available kits (Mfg. by Span Diagnostics Pvt. Ltd , Udhana, India).

Statistical analysis

The results from all the groups at various necropsy intervals were expressed as mean ± standard error of the mean (SEM) to evaluate whether the mean of the sample drawn from experimental (ASE treated irradiated) deviated significantly from respective control (irradiated without ASE). Student's't' test was used by the method of Bourke *et al.*, (1985) [17]. The significance level was set at different levels as $p<0.05$, $p<0.01$ and $p<0.001$.

Results

Acid phosphatase

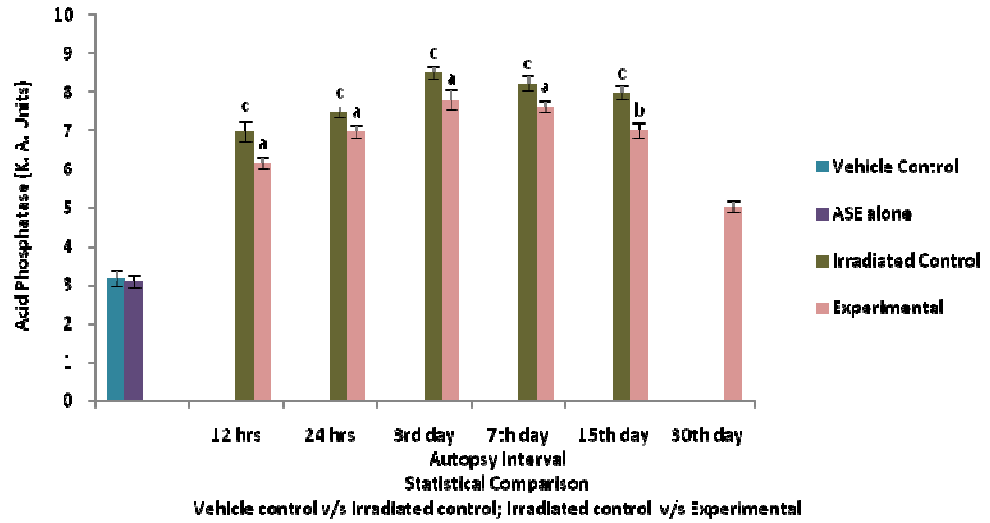
In irradiated control mice (Group-III), serum acid phosphatase level exhibited an increasing pattern above normal from 12 hrs. (6.98 $\pm$ 0.27) to day 3rd (8.50 $\pm$ 0.18) of irradiation. Thereafter, the decline was observed in acid phosphatase level but value could not come down to normal till the survival (i.e. day 15th; 7.98 $\pm$ 0.19) of the animals. This activity was found to be significantly lowered in the ASE treated irradiated mice (Group- IV) from 12 hrs. (6.16 $\pm$ 0.16; $p \leq 0.05$) to day 15th (7.01$\pm$ 0.20; $p \leq 0.05$), however, normal level was not regained till the last autopsy interval (Fig -1).

Alkaline Phosphatase

Serum alkaline phosphatase activity in irradiated control animals (Group-III) followed an apposite pattern to acid phosphatase as initially values declined significantly ($p \leq 0.001$) from first autopsy interval i. e. 12 hrs. (44.58%) and continued up to day 3rd (54.10%) as compared to vehicle treated control. Thereafter, an increasing trend in the activity of such enzyme was noted until day 15th , however no animal could survive beyond this day.

In experimental mice (Group-IV), a decreased level of alkaline phosphatase was recorded till day 3rd (6.01 ± 0.18, $p \leq 0.001$) as in the radiation alone group, but the values were noted to be comparatively higher in this group. On subsequent intervals, an increased level of such enzyme was observed which could not reach to normal even on the last autopsy interval. ASE pre-treatment significantly elevated ($p \leq 0.001$) the alkaline phosphatase level in this group at all the autopsy intervals as compared to corresponding irradiated control (Fig -2).

Fig 1: Variations (mean±S.E.) in serum acid phosphatase activity (K. A. Units) in the peripheral blood of mice after exposure to 7.5 Gy gamma radiation with (Experimental) or without (Control) *Alstonia scholaris* extract (ASE)

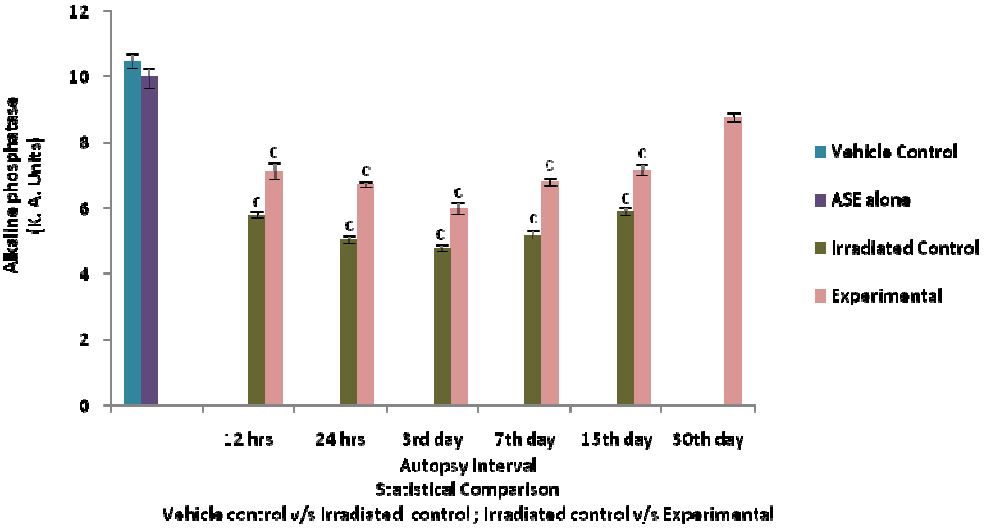


Statistical Comparison

Vehicle control v/s Irradiated control; Irradiated control v/s Experimental

Significance level: a=p≤0.05, b=p≤0.01, c=p≤0.001

Fig 2 : Variations (mean±S.E.) in serum alkaline phosphatase activity (K. A. Units) in the peripheral blood of mice after exposure to 7.5 Gy gamma radiation with (Experimental) or without (Control) *Alstonia scholaris* extract (ASE)



Statistical Comparison

Vehicle control v/s Irradiated control ; Irradiated control v/s Experimental

Significance level: c=p≤0.001

Discussion

Ionizing radiation act either directly or by secondary reactions to produce biochemical lesions that induce a variety of physiological dysfunctions in various organ systems. When ionizing radiation act on solutes dissolved in water then these may result in a number of oxidative events by the products of ionization of water, such as oxidation of sulfhydryl groups, among the enzymes that require their presence for biological activity. It is, therefore, reasonable to assume that these enzymes may be preferentially inhibited on irradiation through oxidation of their sulfhydryl groups to the disulfide [18].

In the present investigation, the activity of serum acid phosphatase (ACP) was found to be increased significantly beyond normal level in the radiation treated groups as compared to vehicle treated control. It is already known that radiation enhances the permeability of membranes of several cellular organelles, which in turn is responsible to enhance the acid phosphates level in blood. A similar increase in acid phosphatase activity after irradiation in different tissues of mice has been reported by various investigators [19-23].

Acid phosphatase is localized in cellular lysosomes and changes in the activity of lysosomal enzymes take place following whole – body irradiation. An enhanced Golgi activity and peroxidation of lysosomal membranes after irradiation causing lysis of membrane and oozing out of the enzymes which are attributed to an increased acid phosphatase level [24]. The discharge of enzymes from lysosomes may be due to activation of pre-existing latent enzymes or due to synthesis of new lysosomes as a consequence of irradiation [25] .

In experimental groups (ASE pretreated irradiated), a significantly lesser activity of ACP towards normal was observed as compared to irradiated control which suggests that ASE may help in causing early recovery by the rapid removal of cellular debris from the tissue collected as a result of radiation damage. These findings are in favor of earlier workers who have also reported significant inhibition in the radiation-induced elevation of ACP in mice using *Mentha piperita* [26] and *Aloe vera* leaf extract[27].

The observed decline in serum alkaline phosphatase (ALP) in the irradiated group in the present investigation may be due to an early decline in the intestinal alkaline phosphatase isoenzyme activity [28]. This decrease may also be attributed to a transitory reduction in the release of alkaline phosphatase to the enzymatic circulation by rapidly metabolizing cells [29], and/or injury to the intestinal mucosa after irradiation as mentioned by Fahim *et al* [30]. The similar observations regarding the decline of ALP after gamma irradiation has also been observed by some of the other workers [8, 23, 27, 31].

The amelioration in alkaline phosphatase activity resulting from ASE before irradiation may be due to favorable alterations in membrane permeability leading to the maintenance of its higher level in serum [32]. *Alstonia scholaris* extract has also been found to inhibit radiation-induced lipid peroxidation and the decline in GSH levels in irradiated mice in our previous study [33]. The depletion of intracellular glutathione (GSH) has been implicated as one of the causes of radiation-induced damage, while increased levels of

intracellular GSH are responsible for a radioprotective action. Increase in GSH and decrease in lipid peroxidation by ASE may be responsible for the maintenance of the optimum level of acid and alkaline phosphatse activity in blood serum.

The protection afforded by *Alstonia scholaris* bark extract against radiation-induced biochemical alterations in phosphatases of serum in the present study may prove to be beneficial for the clinical use of such medicinal plant as a radioprotector.

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