PROTECTIVE EFFICACY OF *ROSEMARINUS OFFICINALIS* EXTRACT AGAINST RADIATION-INDUCED BIOCHEMICAL ALTERATIONS IN MICE

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Summary

Rosemary has long been known for its therapeutic uses. The protective efficacy of rosemary leaves extract (1000 mg/kg b. wt./ day/ animal) was evaluated in terms radiation-induced biochemical alterations in total proteins, cholesterol, acid phosphatase and alkaline phosphatase in intestine. For this purpose, adult Swiss albino mice were exposed to 3 Gy gamma radiation in the presence (experimental) or absence (irradiated control) of *Rosemarinus officinalis* extract (ROE). In irradiated control group, the protein and cholesterol levels were found to be decreased from the normal. Pre supplementation of ROE for 5 consecutive days showed increased levels of protein and cholesterol both. On contrary, radiation alone treatment significantly increased the acid and alkaline phosphatases activity from the normal in group III; whereas, in ROE pretreated irradiated animals, the activity of such enzymes was found to be significantly decreased as compared to the irradiated controls. The exact mechanism of action of this drug at is not known but different antioxidants present in this plant extract may be responsible to scavenge the radiation-induced free radicals, thereby modulating the biochemical constituents of intestine favorably. Thus, the results from the present study suggests the prophylactic use of such plant extract against irradiation.

Key Words: Gamma radiation, *Rosemarinus officinalis*, Swiss albino mice, intestine, protein, cholesterol, acid phosphatase, alkaline phosphatase

Introduction

The gastro-intestinal system is generally recognized as being one of the organ system most readily affected by ionizing radiation. Many of the manifestations such as anorexia, nausea, vomiting, diarrhea and weight loss, may be related to damage to this system. Both anatomical and functional changes in this system have been observed after irradiation. Several detailed studies concerned with histological changes which make it appear that the cells of the gastrointestinal mucosa are very sensitive to radiation.

It has been considered that radiotherapy for cancer patients could be improved by the use of radioprotectors to protect normal tissue. But synthetic protectors, so for tested are found to be toxic at their effective dose levels, which limit their clinical application. Therefore, several studies have been carried out with several herbal preparations such as *Pododphyllum*[1], *Ocimum sanctum*[2], Triphala[3], *Emblica officinalis*[4], *Aegle marmelos*[5] to modify radiation response without being toxic at their effective dose levels. These herbal preparations and plant extracts contain ample quantity of vitamins (A, C, E), carotinoids, flavonoids, enzymes and minerals (Se, Zn, Cu, Mn) and most of the animals including man depend on diet for these antioxidant nutrients. Herbal perpetrations and extracts are gaining importance as less or nontoxic nutritional antioxidants and radioprotectors and several of them are being tested for biochemical and pharmacological properties.

The evergreen shrub *Rosemarinus officinalis* (Rosemary), belonging to family labiatae, is indigenous to southeren europe, and grows widely at north and south coast of mediterranean sea, and also in the sub-himalayan areas. It is used as an antispasmodic in renal colic and dysmenorrheal, in reliving respiratory disorders and to stimulate growth of hair. Extract of rosemary relaxes smooth muscles of trachea and intestine, and has choleretic, hepatoprotective and antitumorigenic activity[6, 7]. It is reported to have anti-inflammatory and antimicrobial activities[8, 9].

The small intestine is a constantly renewing tissue, continuously replacing cells that are lost in the lumen of the intestine. An important function of the intestinal stem cells is to regenerate the tissue after injury. Furthermore, intestine has valuable quantitative parameters as well as serially arranged qualitative cellular configuration. As far as our knowledge no attempt has been undertaken to investigate the effect of rosemary extract on the radiation-induced changes in the gastro-intestinal epithelium of mice. Therefore, present study was undertaken to evaluate the effect of leaf extract of rosemary on the Biochemical alteration in the small intestine of mice exposed to different doses of gamma radiation.

Materials and Methods

Animal care and handling: Adult male Swiss albino mice (6-8 weeks old), weighing 22±2 gm from inbred colony, were used for the present study. They were maintained under controlled conditions of temperature and light (14 and 10 hrs. of light & dark, respectively).The animals were provided standard mice feed (procured from Ashirwad industries, Chandigarh, India) and water *ad libitum*. Tetracycline water was given as preventive measure against infection once a fortnight. Four animals were housed in polypropylene cages containing paddy husk (procured locally) as bedding throughout the experiment. Animals care and handling were performed according to the guidelines issued by the world health organization (Geneva, Switzerland) and the Indian National Science Academy (New Dehli, India). The departmental animal ethical committee approved this study.

Irradiation: The Cobalt teletherapy unit (ACT-C9) at Cancer Treatment Center, Radiotherapy Department, SMS Medical Colleage & Hospital, and Jaipur was used for irradiation. Unanesthetized mice were restrained in a well ventilated Perspex box and whole body exposed to 3 Gy gamma radiation.

Preparation of the *Rosemarinus officinalis* extracts (ROE): *Rosemarinus officinalis* was identified (Voucher specimen No. DDC/2000/DEPTBT) by a well known botanist of Botany Department, University of Rajasthan, Jaipur. Leaves of this plant were cleaned, shade dried, powdered and extracted. The extract was prepared by refluxing with double distilled water (DDW) for 36 hrs (12 hrs x 3). The cooled liquid extract was concentrated by evaporating its liquid contents so as to make it in powder form. An approximate yield of 22% extract was obtained. The extract was dissolved in DDW just before oral administration in mice.

Selection of optimum dose of ROE: Dose selection of *Rosemarinus officinalis* (ROE) extract was done on the basis of our previously[10] conducted drug tolerance and animal survival study .Various doses of ROE (100, 200, 400, 800, 1000, 1500, 2000 mg/kg b.wt/day) for 5 consecutive days were tested against gamma irradiation (8 Gy) against radiation-induced sickness and mortality. The optimum dose (1000mg/kg b.wt./day) thus obtained was used for further experimentation.

Experimental design

Mice selected from inbred colony for the present experiment were divided into following four groups.

Group I (n=21): These animals were given orally DDW equivalent to the dose to ROE (i.e. 1000 mg/kg b.wt/day) for five consecutive days and were considered as Vehicle treated control/Sham irradiated.

Group II (n=21): Animals of this group were administered ROE alone orally by oral gavage once in a day for 5 consecutive days to serve as positive control.

Group III (n=42): These animals given orally DDW equivalent to the dose of ROE (i.e.1000mg/kg b.wt./day) for 5 consecutive days. 30 minutes after the last administration on day 5th, these were exposed to 3 Gy gamma radiation. This group served as irradiated control.

Group IV (n=42): Animals of this group received only ROE (1000 mg/ kg b.wt./day) for 5 consecutive days and were exposed on day 5th with the similar dose of radiation as in group III to serve as experimental.

Animals of all the groups were monitored daily for weight change, behavioral changes, mortality, morbidity, sickness and food and water consumption till their sacrifice or survival. Animals from all these group were sacrificed by cervical dislocation at 12 hrs, 24 hrs, 3rd, 5th, 10th, 20th and 30th days after different treatment. A part of small intestine (as jejunum) was surgically removed and fixed in Bouin'fluid. The tissue was embedded in paraffin block after dehydrating with increasing concentration of 70, 90 and 100 % ethanol. Five micrometer sections were cut using hand microtome, were placed on glass slide and were stained with Harris hematoxyline and Eosin. Stained tissue sections were observed under light microscope to determine histopathological changes.

Biochemical estimation

1. Protein

Total protein content in intestine was estimated by Lowry *et al*. (1951) method in which proteins are precipitated with trichloroacetic acid and folin ciocatteu reagent (phosphotungstic phosphomolybdic acid) and absorbance was read at 640 nm wavelength.

2. Cholesterol

Cholesterol content in tissue was measured by Leiberman Burchard method as given by King and Wolten (1959). Optical density of cholesterol and standard were read at 540 nm wave length.

3. Acid and alkaline phosphatases

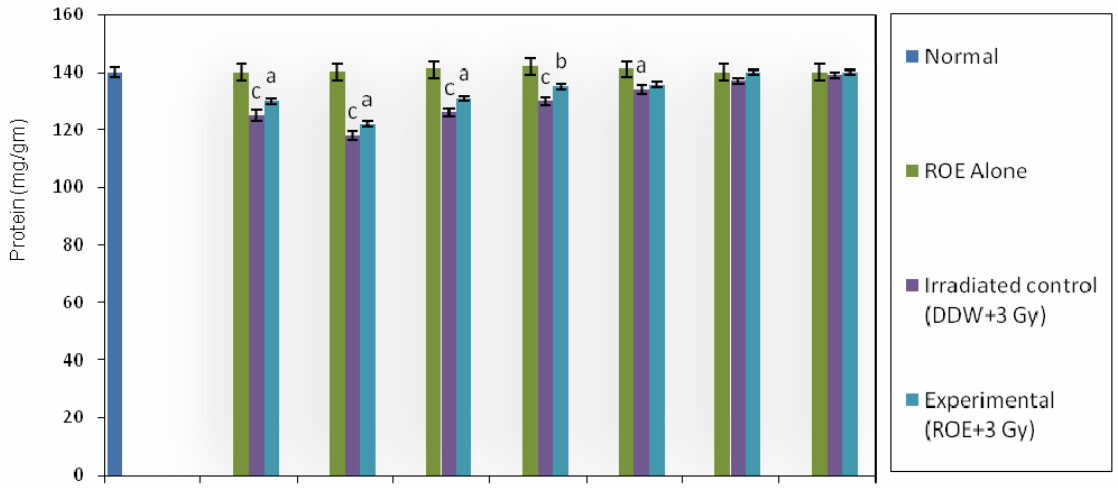
Acid and alkaline phosphatases were estimated by Fiske and Subbarow method given by Hawk *et al.* (1965).

Results

(a) Proteins

No significant difference was observed in ROE alone treated animals with respect to normal (DDW treated) group. In irradiated control animals, protein level decreased significantly ($p \leq 0.001$) till the 5th day of autopsy interval as compared to the normal ones, however, from day 10th no significant difference was noticed in protein content. The protein level was found to be higher in the experimental group (ROE + 3Gy) as compared to irradiated control till the last autopsy interval i.e. day 30 (Fig. 1).

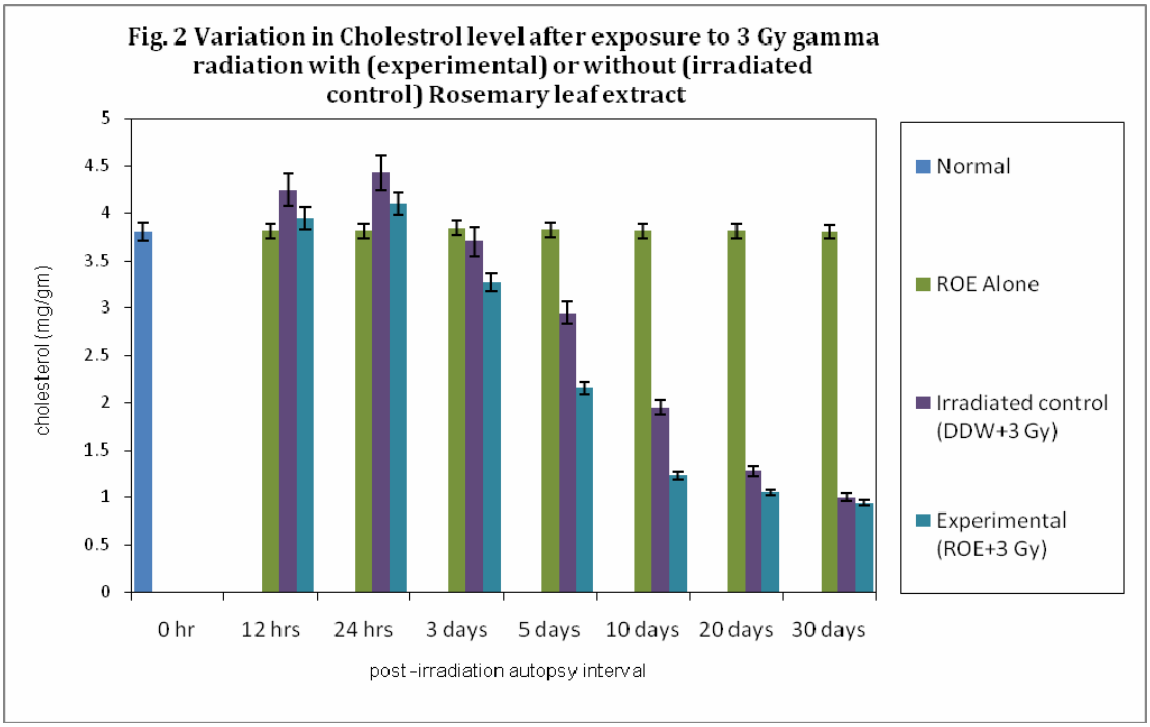
Fig. 1 Variation in Protein level after exposure to 3 Gy gamma radiation with (experimental) or without (irradiated control) Rosemary leaf extract



p values (Normal v/s irradiated control ; irradiated control v/s experimental) : a ≤ 0.05, b ≤ 0.01, c ≤ 0.001

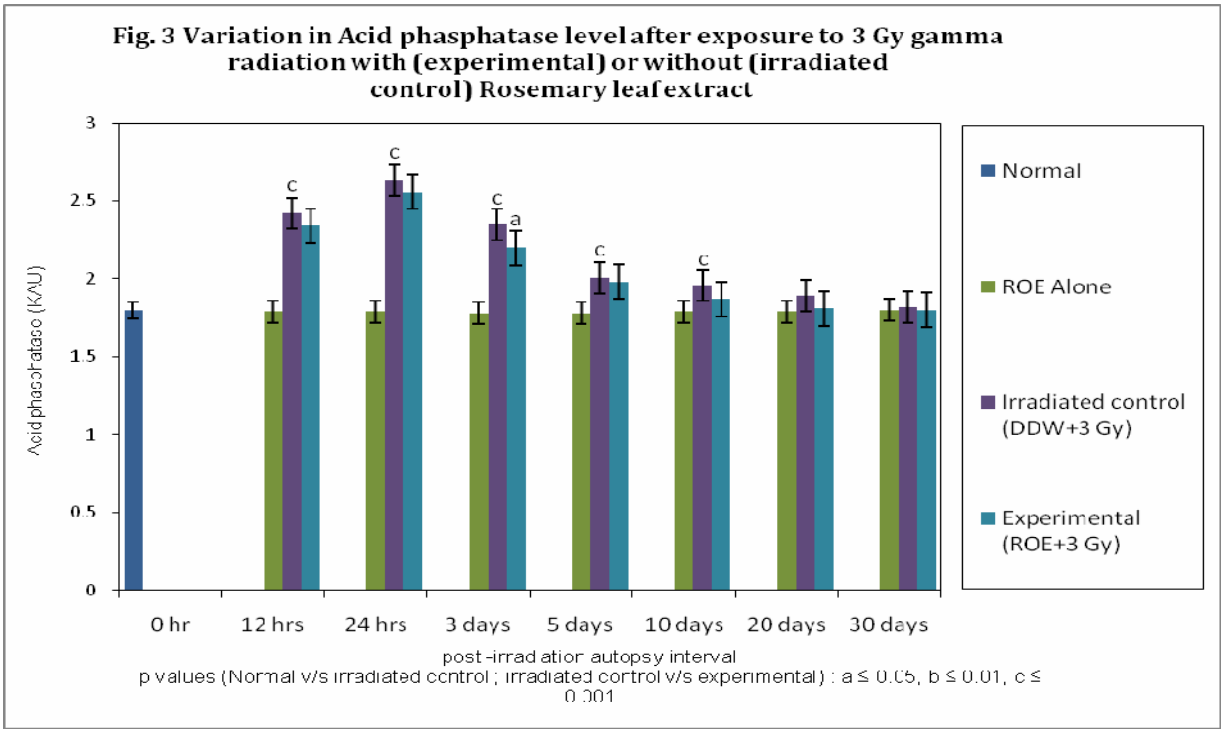
(b) Cholesterol

Almost normal level of cholesterol was recorded in ROE alone treated group. A moderate decrease in cholesterol was observed in irradiated control as compared to the normal animals throughout the experimentation. A slight elevation in cholesterol level was noted in ROE pretreated irradiated group than the irradiated control (Fig. 2).



(c) Acid phosphatase

The acid phosphatase (ACP) activity in intestine was found almost similar in ROE treated group as compared to the Group I. A significant increase in ACP level was observed in the irradiated animals than the normal group throughout the experiment except day 20 and 30. In ROE pretreated irradiated animals, radiation-induced increase in ACP activity was significantly inhibited at all the autopsy intervals in comparison to irradiated control (Fig. 3).



(d) Alkaline phosphatase

Alkaline phosphatase (ALP) activity showed no significant change in ROE treated mice as compared to the Group-I. In irradiated group, the such activity in intestine exhibited a significant ($p \leq 0.001$) increase than the normal ones till day 10^{th} but after which no significant difference was recorded in the same group of animals. In ROE treated irradiated mice, a significant decrease in alkaline phosphatase activity throughout the experimentation was observed as compared to irradiated control. The alkaline phosphatase level was found maximum on day 3 but later a significant decline was noted till the 20^{th} day of post-irradiation and almost normal value was regained on the the last autopsy interval (i.e. 30^{th} day) (Fig. 4).

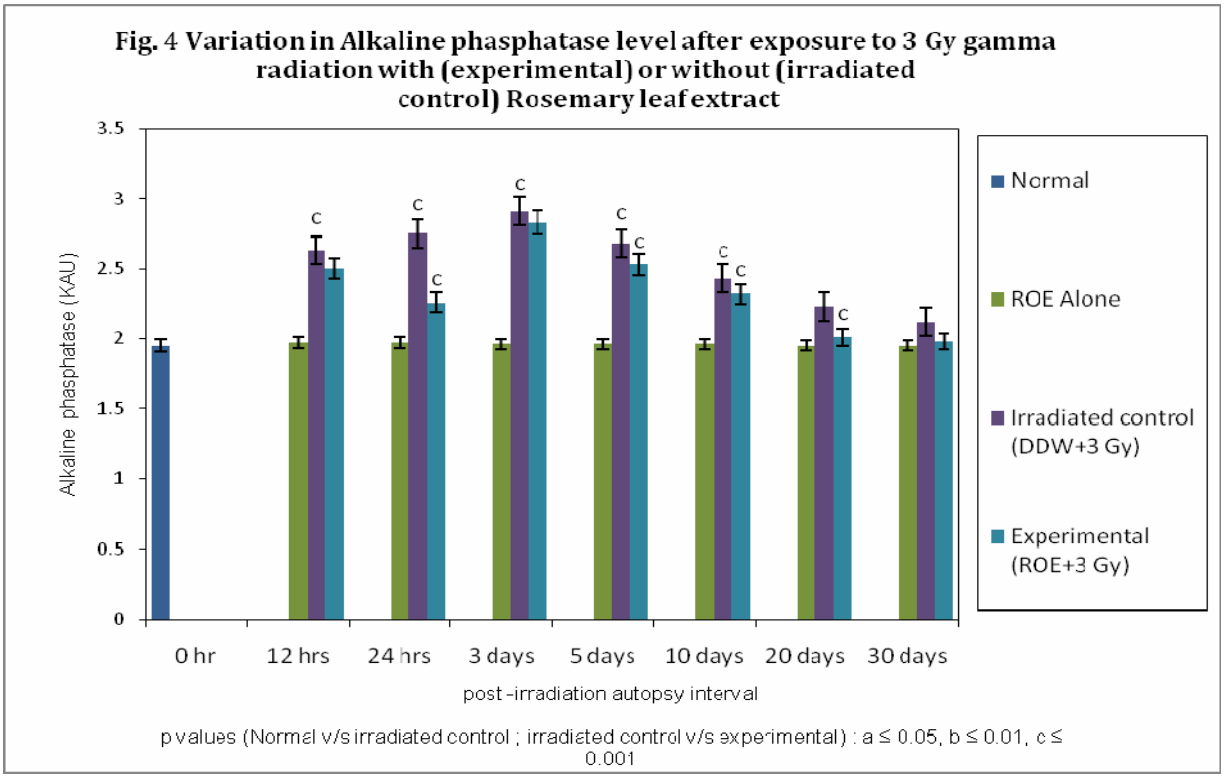


Fig. 4 Variation in Alkaline phasphatase level after exposure to 3 Gy gamma radiation with (experimental) or without (irradiated control) Rosemary leaf extract

p values (Normal v/s irradiated control ; irradiated control v/s experimental) : a ≤ 0.05, b ≤ 0.01, c ≤ 0.001

Discussion

The present study reveals that the irradiation of animals to 3 Gy gamma rays resulted into mild radiation sickness within 3-5 days of radiation exposure. The symptoms included reduction in the food and water intake, weight loss and fluid loss by diarrhea and diminished absorption capacity of gastro-intestinal tract. The similar symptoms have been observed in mice after irradiation by others also [14, 15]. On contrary, ROE treated irradiated mice did not show any radiation induced sickness and adverse symptoms.

The present study revealed that after exposure to 3 Gy, the proteins level was found to be decreased up to 24 hrs., thereafter, a considerable increase was observed until the last autopsy interval, and the similar trend was observed in the earlier study also [16]. Total proteins content in intestine was significantly higher in ROE treated group at early interval. Such rise in protein may be due to an increase transport of amino acids through plasma membrane as a consequence of permeability change in irradiated cell membranes in the experimental group. Increase in protein concentrations, as compared to irradiated control, with supplementation of various medicinal plant extract after gamma irradiation has already been reported by the others[17, 18].

The cholesterol level in Group III, was found to be considerably decreased at all the autopsy intervals as compared to the normal animals, and the same was also noted by others[19]. In irradiated experimental animals, cholesterol level was considerably lower at 12 hrs. but after that it increased gradually and regained the normal level by the next autopsy interval. These observations substantiate with early findings of other investigators [17, 20] where an increased plasma cholesterol level was noted in mice after irradiation.

Acid phosphate enzyme is localized in cellular lysosomes, and its activity may be changed following to whole-body radiation. An elevated Golgi activity and peroxidation of lysosomal membranes after irradiation causing lysis of membrane, and oozing out of this enzyme is attributed to an increased acid phosphates level [21,22]. The enhanced activity of acid phosphates found in other tissue like intestine could be ascribed either to a direct effect of irradiation on the lysosomal membrane or some indirect effect such as liberation of thyroid hormone [21].

The present study indicated alterations in the activities of some key enzymes that reflect the radiation- induced oxidative stress. In irradiated alone treated animals (Group III), the acid phosphatase level was found to the highest at 24 hrs post-irradiation and thereafter declined gradually with post-irradiation time, and this observation is in a good agreement with findings of others [23,24]. On the other hand, in ROE pretreated irradiated mice (Group IV), a considerably lower level of acid phosphatase were observed as compared to their respective irradiated control. An increasing value of acid phosphatase enzyme in intestine was observed up to 24 hrs., thereafter, it declined progressively and reached to normal level on 30th day of irradiation. A significant increase in acid phosphatase activity in various tissues of Swiss albino mice exposed to different doses of gamma radiation has been noticed by some of the investigators [24, 25, 26].

Alkaline phosphatase plays an important role in maintenance of cell permeability and acts or mono – phasphatases. Damage to cell membrane caused by radiation may be the reason for declined activity of such enzyme after irradiation. In irradiated control group, alkaline phosphatase activity increased significantly till day 3rd, and after that the same declined gradually till the last autopsy interval. This decrease may be attributed to the severe damage to GI tract. In Group IV (ROE +3 Gy), alkaline phosphatase activity was found to be much lowered as compared to the irradiated control. It was found as maximum on day 3rd, followed a decline and restored the normal levelby the end of the experimentations (i.e.30 days). The decrease in alkaline phosphatase activity in mammals after gamma irradiation has also been observed by some other workers [20, 27, 28]. Acid and Alkaline phosphatase are the enzymes, concerned with biosynthesis of fibrous proteins[29] and mucopolysaccharides[30]. They also act as the hydrolytic enzymes which play an important role in dissolution of dead cells of the body [31].

The exact mechanism of action of rosemary is not known; however, it may scavenge radiation induced free radicals and thus reduces damage to the cellular DNA. There are six main polyphenolic compounds present in rosemary extract as carnosic acid, carnosol, 12-O-methyl carnosic acid, rosemarinic acid, genkwanin and isoscutellarein 7-O-glucoside [32]. Hydro-alcholic extracts of some medicinal plants with high amount of hydroxycinnamic derative content of which RA present as more than 3-6% of the dry wt. Were tested and have shown significant anti oxidative activities by free radical scavenging effect on DPPH. From the promising results obtained in the present study, it can be anticipated that *Rosemarinus officinalis* leaves extract has the potential to protect individual against radiation induced oxidative stress and intestinal lesions.

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