Whole body protection to lethally irradiated mice by oral administration of semipurified fraction of *Podophyllum hexandrum* and post irradiation treatment of *Picrorhiza kurroa*

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Objective: **To evaluate the radioprotective potential of alcoholic fraction of *Podophyllum hexandrum* rhizomes (REC-2001) individually as well as in combination with *Picrorhiza kurroa* administered orally in lethally irradiated Swiss albino mice.**
Methods: **The study was divided into different treatment groups. Whole body survival was observed upto 30 days in all the treatment groups. Besides survival, toxicity of REC- 2001 was also evaluated. All the groups were studied for spleen endogenous colony forming units (CFUs), plasma antioxidant potential and hematological variables, using standard techniques.**
Results: **Animals in radiation alone group died with in 12 days of exposure. Single dose of REC-2001 which did not bring any toxic manifestation/mortality (MTD) was found to be 155 mg/kg b.w. On administration of 250 mg/kg b.w. (single dose) 50% of the animals died (LD50), while a dose of 350 mg/kg b.w. of REC-2001 brought 100% death. Oral administration of single dose of REC-2001 (25mg /kg b.w. -1h) prior to irradiation (10Gy) was observed rendering up to 48 % protection. Survival enhanced to the level of 55% when the animals had pre- treatment of REC-2001 (25mg /kg b.w. -1h) followed by irradiation (10Gy) and post treatment with a single dose of *Picrorhiza kurroa* rhizome extract (pkre, 8mg/kg b.w.+1h). Radiation induced plasma antioxidant status was significantly (P<0.02) countered by REC-2001 administration. Post treatment of pkre elevated CFU counts (P<0.05) .Total leukocytes count and hemoglobin content in REC-2001 pretreated and pkre post treated group approached normal limits within 30 days of the study.**
***Conclusion*: REC-2001 in combination with pkre holds promise for further studies to achieve radioprotection against lethal radiation by oral administration.**

Key words: Radioprotection, *Podophyllum hexandrum, Picrorhiza kurroa,* Whole body survival, Lethal radiation, Herbal radioprotector.

INTRODUCTION

Fear of nuclear accidents and nuclear terrorism is ever increasing due to increased applications of nuclear technology [1]. In nuclear accident scenario every individual needs personal and specialized attention due to large probability of radiation contamination, immediate and long-term affects of radiation, transfer of mutations from generation to generation and retention of long lived radionucleids in the body besides, various other invisible factors [2]. The appropriate remedy for such situations known so far is either prevention or protection against the source. Protection could be physical/biological or a conglomerate of both. Physical protection system generally collapses in nuclear disaster scenario and finally major dependency for saving human lives has to be on biological protection.

Five decades of research in the field [3] unfortunately have led to no satisfactory outcome. The reasons behind this could be many besides the toxicity of the presumptive radioprotector. Plethora of synthetic compounds, mainly those containing thiol groups have been studied extensively [4] to protect biological system against radiation. However, only a few of the synthetic preparations could find limited clinical acceptance due to their inherent toxicity at therapeutically relevant doses [5].

Herbal preparations are in use to treat enumerable diseases [6, 7] since ancient time. Their clinical use is increasing due to better understanding of the chemical nature of the bioactive principles present in them. *Podophyllum hexandrum* (family: Berberidaceae) thrives in the Himalayan region at 3000–4000 meters altitude. Roots and rhizomes of this plant have been used in *Ayurveda* against various ailments like nervous disorders, viral and bacterial infections, genital warts, leukemia, Hodgkin's and non-Hodgkin's lymphoma, constipation, common cold etc.[8]. Fractions prepared from rhizomes of *P. hexandrum* were studied for the first time in our laboratory for its ability to render radioprotection. *In-vitro, in-vivo and ex-vivo* studies carried out so far revealed the potential of this plant extract

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to protect against radiation induced mortality [9–11]. Up to 90% survival against lethal Y-irradiation was achieved in Swiss albino mice using the plant fractions [12–14]. However, the studies conducted so far used the intra peritoneal (i.p) route for extract administration. Unfortunately i.p route had never been a route of preference for drug administration particularly in case of mass human exposures.

The present study, for the first time, reports the efficacy of *P. hexandrum* fraction as a radioprotector when administered orally in mice model. Though oral route of drug administration is widely preferred, it rendered less efficacy compared to i.p route as discussed later. *Picrorhiza kurroa* (family: Scrophulariaceae), a small perennial herb growing at an altitude of 3000 to 5000 meters in Himalayas have been used in traditional Ayurvedic system of medicine to treat liver troubles, bronchial disorders, dyspepsia, bilious fever, chronic dysentery and scorpion sting [15, 16]. The roots and rhizomes of this plant are found to contain active molecules such as picroside and kutkoside, apocynin and androsin [15]. This plant since is known to help boost immune system was considered as a means of post irradiation care. Administration of pkre (*Picrorhiza kurroa*) one hour post irradiation was shown to increase survival as well as improve health status of all the animals.

MATERIALS AND METHODS:

Collection of plant material (*P. hexandrum)*:

Rhizomes of *P.hexandrum* Royale were collected from Jammu and Kashmir, India. The material was taxonomically identified at Regional Research Laboratory (RRL), India. The voucher specimen was deposited at RRL, Jammu with a Voucher No. RRL/Ph/Srinagar 2005.

Method of Extraction (*P. hexandrum)*:

Extraction was performed following the technique described by Sagar *et al*, 2006[17] and Gupta *et al*, 2007 [13]. In brief, collected sample of the plant rhizome was washed thoroughly with running water and dried under normal conditions. The dried sample was powdered and subjected to the extraction with solvent of increasing polarity. Podophyllin was precipitated using 1% HCl. The extract was vacuum dried and dry resin was refluxed in Soxhelet apparatus using petroleum ether followed by chloroform for extraction. The chloroform soluble extract was dissolved in methanol and boiled with neutral alumina. The solution was filtered and evaporated in a vacuum rotavapor. Left over residue was dissolved in methanol and benzene to crystallize the podophyllotoxin. The mother liquor left after removal of major amount of podophyllotoxin was concentrated and coded as REC- 2001.

Preparation of Picrorhiza kurroa extract (pkre):

After proper identification and authentication of plant material by a botanist, the finally prepared extract from rhizomes of *Picrorhiza kurroa* was obtained from RRL Jammu. For extraction, the rhizomes were air dried and powdered in a mechanical grinder. After passing through various extraction processes the residual solvent was removed under vacuum in a rotary evaporator. The final extract was stored until the completion of studies in a desiccator at 4°C.

HPLC analysis of *Podophyllum hexandrum* extract

Isocratic analysis:

REC-2001 was analyzed on Shimadzu LC-10 AT/VP HPLC machine isocratically, using E. Merck RP-18 e column (250x4.0 mm 5µm) with diode array detector SPD M-10 A VP/RF-10 AXL fluorescent detector and auto injector SIL-10 AD VP. Elution was done with the mobile phase (MeOH: H20; 60:40) for 25 minutes at a flow rate of 0.8 ml/min and a wavelength of 290 nm was used for measurement.

Gradient analysis

The compounds were analyzed on Shimadzu LC-10 AT/VP using a diode detector with mobile phase consisting of methanol (A) and water (B), 0.01–5 min (A: B; 65: 35), 5–65 min (A: B; 35: 65) and finally 65–75 min (A: B; 65: 35), E. Merck RP-18 e column (5µm, 4x250 mm). Separation was carried out at 300°C with a flow rate of 0.6ml/min. Measurements were taken at 290 nm. Characterization of active constituents was carried out by co-spiking with standard markers and using LC-MS and LC-MS/MS. Lignans were quantified by standard calibration. The qualitative analysis of three flavonoids viz., quercetin, kaempferol and kaempferol-3-glucoside was done by comparing their TLC, HPTLC and HPLC profiles with reference standard markers [17].

Experimental design:

Animals and different treatments:

Swiss albino male mice (8–10 weeks old, 28 ± 2g) were kept under standard animal house conditions and fed on standard animal food and water *ad libitum.* All experiments were conducted as per the guidelines of the Institute Animal Ethical Committee (IAEC). Each group had 6 animals and most of the experiments were repeated three to four times. Animals for survival studies were divided into 6 groups: normal control group (without any treatment), in radiation control group (animals exposed to 10 Gy whole body γ-irradiation). REC-2001 (25 mg/kg b.w, -1 h) prior to 10 Gy whole body γ-irradiation (WBI) was given in another group. Group 4 received REC-2001 (25 mg/kg b.w) 1 h prior to 10 Gy whole body γ -irradiation and post treatment of pkre (8 mg/kg b.w, +1 h). Different doses of REC-2001 (Group 5) and pkre (Group 6) were administered in separate groups of animals to observe their independent effects on survival of un-irradiated mice.

Maximum tolerable dose and Toxic doses (MTD, LD50, LD100):

The amount of REC-2001 which did not bring about any death/toxic manifestations in the experimental animals was considered as MTD. The doses causing 50% and 100% mortality were defined as LD50 and LD100 respectively. It was not felt imperative to conduct toxicity studies with *Picrorhiza kurroa* as roots and rhizomes of this plant are very commonly used in *Ayurveda* as immune enhancer in multiple and

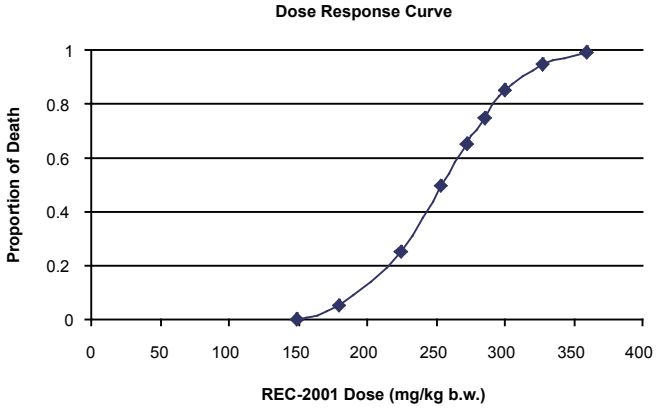

Fig. 1 Screening of different doses of REC-2001 for toxicity. Different concentrations of REC-2001 were administered orally. As per the fitted curve MTD, LD50 and LD100 are observed as150, 254 and 350 mg/kg b.w. respectively. Study was repeated thrice with six animals per group.

daily doses while in this study we used only single dose of this plant extract and that too also in small quantity.

Irradiation:

Animals were irradiated in a 60Co γ source (Dose rate 0.51−0.45 cGy/sec). For radiation exposure mice were placed in perforated plexi glass container and irradiated individually under continuous supply of fresh air to avoid hypoxia. Dose rate was calibrated at regular intervals using Baldwin Farmer's secondary dosimeter and Fricke's dosimetry method.

Whole body survival and Body weight:

To observe the effect of REC-2001, pkre and irradiation either individually or in combination, the body weight, physical symptoms like lethargy, edema, epilation and survival in all the experimental groups were recorded every day up to 30 post irradiation days.

Endogenous spleen colony forming unit (CFU) assay:

For endogenous CFU assay, mice were sacrificed by cervical dislocation on 10th post treatment day. Spleen from all the experimental animals were removed and fixed in Bouin's fluid for 24h.The colonies appeared on spleen, visible to the naked eyes were scored individually.

Antioxidant Potential:

Ferric Reducing Activity of Plasma (FRAP assay)

The antioxidant potential in terms of ferric reducing activity of plasma was studied as described earlier [10]. In brief, plasma separated from the blood drawn from the heart of each animal was diluted with water in 1: 4 ratio and mixed with 200ml of FRAP solution (FRAP solution: 25ml acetate buffer+2.5ml TPTZ+2.5ml ferric chloride hexahydrate). The solution was incubated for 10 minutes and OD was taken at 593nm. Standard curve was plotted using 100 to 1000µl $FeSO_4.7H_2O$.

Hematological studies:

Total leucocytes count (TLC) and hemoglobin concentration were estimated manually in peripheral blood drawn from the heart of mice at different time intervals by using standard techniques.

Statistical Analysis

Whole body survival data was analysed by Kaplan Meier survival curves and Log Rank test. Finney's Probit Analysis was used for toxicity studies. FRAP assay and hematological values were analysed using ANOVA. Spleen colony count statistics was done by using Mean and standard deviation.

RESULTS

HPLC analysis of REC-2001 indicated the presence of lignans and flavonoids. The major lignans identified were Podophyllotoxin, Podophyllotoxin 1-0-β-D glucopyranoside, Isopicropodophyllotoxin, Deoxypodophyllotoxin and Picropodophyllotoxin. Besides, there were flavonoids including Quercetin, Kaempferol and Kaempferol-3-glucoside.The major molecules identified in pkre (*Picrorhiza kurroa*) were Picroside, Kutkoside, Androsin and Apocynin.

Toxicity:

Oral administration of single dose of REC-2001 up to 155mg/kg b.w was tolerated well by the animals without any indication of adverse symptoms. Therefore, this dose was considered as the maximum tolerable dose (MTD). A dose of 250mg/kg b.w of REC-2001 rendered 50% death where as 350mg/kg b.w brought 100% death of the treated animals within 5 days of administration. From the fitted dose response curve (Fig.1), MTD was estimated to be 150 mg/kg b.w while the LD50 value of the extract corresponds to 254mg/kg b.w at a confidence interval of 95% (239, 263).

Whole body survival assay:

Animals in radiation alone group died within 12 days of exposure (Fig.2). Oral administration of single dose of REC-2001 (25mg/kg b.w) 1h before 10 Gy WBI rendered 48% survival against 100% mortality in radiation control group. The doses of REC-2001 experimented other than 25mg were 15, 20, 30 and 40 mg/kg b.w. The whole body survival with 15 and 20 mg/kg b.w. was 15% and 25% respectively while 30 mg/kg b.w rendered upto 33% survival and the dose of 40 mg/kg b.w. did not show any significant survival in lethally irradiated animals. The most effective concentration of REC-2001 thus obtained (25mg/kg b.w.) was administered at different time intervals i.e. 0.5 h,1h, 2h and 3h before irradiation to estimate the optimal time gap between REC-2001 administration and radiation exposure. The optimal survival obtained

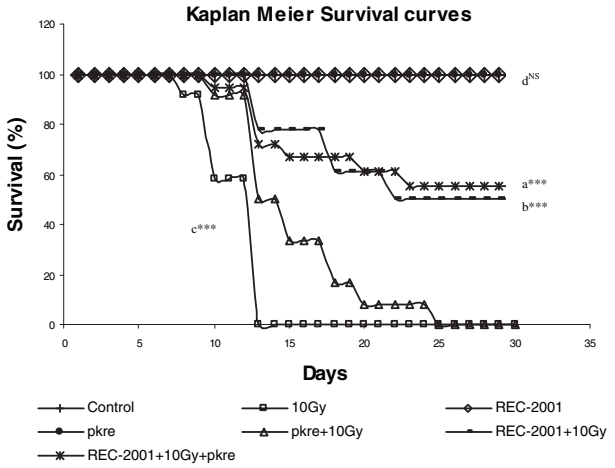

Fig. 2 REC-2001 (25mg/kg.b.w.) was administered orally to Swiss albino male mice 1 h before exposure to 10Gy gamma radiation. Post treatment of pkre (8 mg/kg. b.w.) was given 1 hr after irradiation. Survival was observed for 30 days after exposure. Data was analysed by Kaplan Meier Survival Curves. Experiments were repeated 4 times and each group had six animals

a- 10Gy vs REC-2001+10Gy+pkre, b-10Gy vs REC-2001+10Gy, c-Normal control vs 10Gy, d-Normal control vs REC-2001 and pkre individually. NS-non significant.
*** P<0.001

at 0.5h, 2 h and 3 hrs was 25%, 35% and 25% respectively. A time interval of 1 h was found to be the most optimal as this time gap rendered maximum survival of 48%.

Animals administered with REC-2001 and irradiated thereafter were further treated with *pkre*, the *P. kurroa* rhizome extract. The different concentration of single and multiple doses of pkre were studied at various time intervals and also at pre and post irradiation scenarios. An amount of 8mg/kg b.w. *pkre* administered 1h after irradiation increased the whole body survival to 55% compared to 48% in the irradiated REC-2001 pretreated group. Since 1h post irradiation rendered the best result therefore, hence to be deleted 8mg/kg b.w *pkre* and 1h post irradiation time was considered in all further studies. Multiple dose administration of REC-2001 maximum upto five days (single dose/day) before irradiation could extend the survival of 70% animals up to 22 days. The maximum weight loss observed in radiation control groups was up to 45–60% of original body weight of animals. In REC-2001+radiation group weight reduction was up to 25–30%. While in REC-2001+radiation+pkre group only up to 20% loss of the original weight had occurred and that too also started reverting after 10th day of exposure. The effective doses REC-2001 and pkre individually did not exert any lethal toxicity.

The survival data analysis was carried out using Kaplan Meier survival curves and their significance was studied using Log Rank statistics. The Kaplan Meier curves revealed that REC-2001+10Gy +pkre and REC-2001+10Gy groups had significantly higher survival compared to radiation only group (P=0.00). Though there was no significant difference (P=0.88) between REC-2001+10Gy +pkre and REC-2001+10Gy the former has shown slightly better survival pattern than later. There was no significant difference between normal control group compared to REC-2001 or pkre group individually.

Hematological variables:

In 10Gy WBI group (Table.1), the white blood cell counts marked a sharp decline within 72h of irradiation and that continued till the death of animals (12th post-irradiation day). Though the WBC counts during initial period of study in REC-2001 alone group was also found to reduce as compared to the control animals but it was not statistically significant (P>0.2). There was also no significant difference in the WBC counts between the 10Gy WBI animals and animals treated with REC-2001 before 10Gy WBI up to 72 h (P >0.5). However, on 10th day of the study this difference became highly significant (P < 0.01) following the slow but steady recovery in WBC counts of the REC-2001 pretreated and irradiated animals. The recovery in WBC counts in REC-2001 pretreated animals continued at a constant pace but the values (3.95 ±0.34) still remained lower as compared to untreated control animals (P <0.01) till 30th day of study. In the REC-2001+10Gy +pkre group, the recovery in WBC counts was much faster and the values became comparable to that of untreated control animals by 30th post-irradiation day. On administration of pkre alone there was absolutely no change in WBC counts.

The hemoglobin content remained relatively unchanged in all the treatment groups till 72 h. post-irradiation time (Table.1). On fifth day of study, the difference in Hb concentration between un-irradiated and irradiated group became significant (P <0.01) while on 10th day the gap was highly significant (P <0.001). REC-2001 pretreatment could significantly counter the reduction in Hb content as compared to 10Gy WBI (P <0.01), however, the values remained less as compared to untreated controls till 30th day. On 30th day of study hemoglobin concentration in the REC-2001+10Gy+pkre was comparable to the control group and was significantly higher than REC-2001 pretreated irradiated group. Administration of pkre and REC-2001 individually did not make any change in hemoglobin content at any interval of study.

Endogenous spleen colony forming assay:

In radiation control groups spleen size on 11th day of treatment was about half of the untreated animals and no colony (0 ±0) was observed on spleen of either groups (Table 2). In REC-2001+10Gy WBI treated animals, the average number of spleen colonies was observed to be 05± 0.16 (mean± SD) compared to 15±0.18 colonies in REC-2001+10Gy WBI + pkre. Spleen size of the animals in the later group was also comparable to that of the untreated animals. In REC-2001+10Gy WBI group the average spleen size was

Table. 1 Effect of REC-2001 and REC-2001+ pkre on the total leucocytes count (Mean ± SD) and Haemoglobin (mean ± SD) in peripheral blood of Swiss albino male mice exposed to 10 Gy whole body gamma irradiation. The study was repeated thrice with six animals per study

Groups	Post treatment days			
	3 days	5 days	10 days	30 days
Leucocytes count (X 1000/ mm^3)				
Control	6.06±0.18	6.06±0.20	6.06±0.18	6.06±0.17
10 Gy	0.69±0.12a***	0.15±0.06a***	0. 01±0.25a***	ND
REC 2001	5.43±0.30a ^{NS}	5.45±0.18a ^{NS}	5.29±0.37a ^{NS}	6.15±0.95a ^{NS}
Pkre	6.16±0.08a ^{NS}	5.49±0.06a ^{NS}	5.87±0.15a ^{NS}	6.09±0.81a ^{NS}
REC 2001+10 Gy	0.72±0.07 b ^{NS}	0. 65±0.09b**	0.86±0.10 b**	3.95±0.28a**
REC 2001+10 Gy+pkre	0.77±0.01c ^{NS}	0. 98±0.02c*	1.50± .01c*	4.79±0.15c*
Hemoglobin (g/dl)				
Control	15.17±0.38	15.17±0.09	15.17±0.28	15.17±0.15
10 Gy	14.28±0.36a^{NS}	10.68±0.29a*	6.16±0.97 a***	ND
REC 2001	15.12±0.28a^{NS}	14.42±0.17a^{NS}	14.76±0.27 a^{NS}	14.92±0.18a^{NS}
Pkre	14.22±0.81a^{NS}	15.12±0.31a^{NS}	14.96±1.23 a^{NS}	15.13±0.67a^{NS}
REC 2001+10 Gy	13.60±0.17b ^{NS}	12.86±0.01b*	8.82±0.11b*	9.12±0.95
REC 2001+10 Gy+pkre	13.50±0.27	12.52±0.16	9.48±0.18c**	12.51±0.12c**

*P < 0.05; ** P < 0.01; *** P < 0.001; ND= Not done because of death of animals; a=Compared with control; b= Compared with 10 Gy; c= Compared with 10 Gy+REC-2001.

Table. 2 Endogenous colony counts on spleen of pretreated (REC-2001), irradiated (10Gy) and post treated pkre male mice. The study was repeated thrice with six animals per study.

S. No.	Groups	Colony counts/spleen (Mean±S.D).
1.	Untreated Control	0±0.00
2.	Radiation (10 Gy)	0±0.00
3.	REC-2001 (25mg/kg b.w., -1h +10 Gy	05±0.16
4.	REC-2001 (25 mg/kg b.w., -1h) +10 Gy +pkre (8mg/kg. +1hr)	15±0.18

smaller as compared to that in control animals but was larger than the spleen size in radiation alone group mice (gross observations).

FRAP assay:

10Gy WBI led to significant decrease in Ferric reduction potential of plasma (Fig.3) as compared to control animals. This reduction continued up to 4h interval and hereafter started recovering. At 24h interval values were close to untreated group. REC-2001 pretreatment significantly countered radiation induced depletion in antioxidant potential of plasma. Maximum effect of REC-2001 was seen up to 4h (P <0.01) and beyond this time point the FRAP value in all groups were not statistically different. Post treatment of pkre in REC-2001 pretreated irradiated group sharply increased Ferric reduction potential particularly at 4h intervals (P <0.001) and at later intervals also values remained higher. Treatment of REC-2001 and pkre individually in un-irradiated groups brought in some changes though statistically non-significant in antioxidant potential of plasma at initial stages of treatment (Fig.3).

DISCUSSION

Plant products, due to their synergistic action and comparatively non-toxic nature have been exploited worldwide for their radioprotective potential [18, 19]. Our group too has extensively screened large number of plants [12, 20, 21] for their radioprotective potential. In process, various fractions of *P. hexandrum* have been studied in detail for providing whole body survival and systemic protection against lethal radiation. REC-2001, a fraction prepared from *P. hexandrum*, has been extensively evaluated for its phytochemical details also using HPLC, LC-MS, MS-MS etc [17]. The major lignans in addition to flavonoids present in the plant are well known for their antioxidant potential [22, 23]. Phenolic compounds being rich in hydroxyl groups are capable of scavenging free radicals by forming

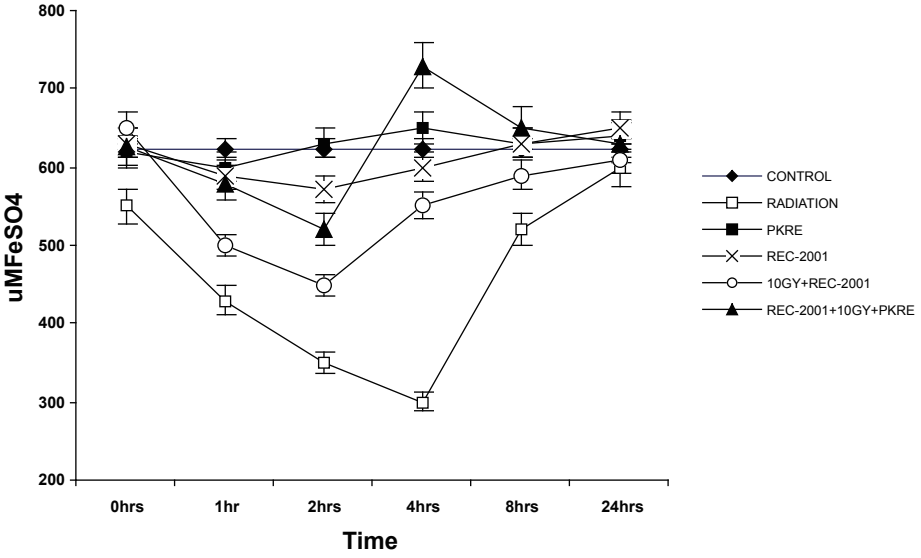

Fig. 3 Ferric reducing ability of plasma (FRAP) in the blood of mice in different treatment groups. Data was pooled and analysed by 4 independent experiments having 4 animals in each groups. Values are expressed as mean ± SE.

resonance stabilized phenoxy and quinine radicals [9].

Intraperitoneal administration of *P. hexandrum* fraction (REC-2001) has rendered up to 90% protection against radiation induced 100% mortality in Swiss albino mice [12, 14]. Any substance when administered orally is known to render reduced efficacy mainly due to poor absorption in GI tract, variations in gut pH and metabolic interference by liver. Still this route is the most preferred due to minimum involvement of operational procedures and less probability of direct toxicity. Our studies have shown up to 48% (Fig.2) protection by REC-2001 on single dose administration by oral route while >90% survival was achieved with lesser concentration of the extract when administered intraperitonealy [12]. Though MTD, LD50 and LD100 doses also increased significantly after oral administration in comparison to intraperitoneal route.

In all the experimental groups white blood cell count reduced gravely during initial days of treatment (Table.1), but in radiation alone group this reduction progressed till the death of animal. While in REC-2001 pretreated +10Gy WBI and the group given REC-2001+10Gy WBI followed by pkre treatment, loss was observed only until few initial days of study. Reduction in WBC fall in afore mentioned groups at later stages might be possible due to free radical scavenging by REC-2001 pretreatment in the beginning leading to minimum cellular damage. Additive support by post treatment with pkre, rendered to the immune system in later stage, has also probably helped in speedy recovery of WBC count. This observation is in consonance with the studies published earlier [24] wherein *P. kurroa* extracts have been found up-regulating the expression of Th1 cytokines IL-2, IL-12, IFN-γ, TNF alpha and Th2 cytokine IL-4.

Sharp decline in hemoglobin content in radiation alone group was noticed on 10th day (Table.1) of irradiation. This could be primarily because of loss of RBC from circulating blood mainly due to hemorrhage and capillary leakage. Reduced supply of fresh RBC in peripheral blood due to severe loss of precursor cells in bone marrow probably added further to this loss. Comparatively less reduction in hemoglobin content in REC-2001 pretreated and irradiated animals confirmed the protection rendered to bone marrow stem cells by REC-2001, which is also in conformity to our previous studies [17]. Further marginal reduction in hemoglobin concentration on post treatment of pkre indicated therapeutic role of *P. kurroa* extracts for hematopoietic system of treated animals. Noticeable increase in number of colonies on the spleen of animals having REC-2001+10Gy WBI followed by pkre treatment in comparison to other three groups, is further a very strong evidence for support rendered by pkre post treatment to the immune system of irradiated animals.

Ferric reduction potential of REC-2001, evaluated during current and in previous studies also [10] clearly indicate antioxidant potential of this fraction. This statement is in consonance with our earlier i*n-vitro* studies indicating significant reduction in hydroxyl radical generation [9] by REC-2001. Preliminary studies conducted to understand the mechanism of protection rendered by *P. hexandrum* against radiation has revealed that the fractions prepared from this plant mainly act by inhibiting Apoptosis Inducing Factor (AIF), up-regulating expression of protein responsible for cell proliferation and modulation of proteins expression associated with cell death in addition to scavenging free radicals to a great extent [14].

Current studies have reveled that though oral administration of REC-2001 has not rendered protection to the extent of intraperitoneal route administration but it is clearly shown that REC-2001 alone can save upto 48% of lethally irradiated mice and post treatment with pkre can further enhance the survival rate

as well as overall health status of the animals against nil survival in radiation alone group**.** The health status of mice was measured in terms of whole body weight, food intake, liver status, hematological variables, spleen size and spleen CFU. The combination was found rendering significant protection to the above mentioned vital organs. However, further detailed studies in this direction are warranted to use this fraction in humans against radiation induced injuries.

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REFERENCES

1) Pellmar TC, Rockwell S. Priority list of research areas for radiological nuclear threat counters measures. Radiat Res. 2005; 163: 115-123.
2) Giambarresi L, Jacobs AJ. Radioprotectants: Military Radiobiology. Conklin JJ and Walker RI (Eds.). Academic Press, Inc, London. 1987; 265-301.
3) Maisin JR. Chemical radioprotection-past, present and future prospects. Int J Radiat Biol 1998; 73: 443-450
4) Bump EA, Malaker K. Radioprotectors: Chemical, Biological and Clinical perspectives. CRC Press, Boca Raton, Florida, 1998; 431.
5) Cairnie A. Adverse effects of WR-2721. Radiat Res 1983; 94: 221-226.
6) Devasagayam TPA, Sainis KB. Immune system and antioxidants, especially those derived from Indian medicinal plants. Ind J of Exp Biol 2002; 40: 639-655.
7) Chatterjee A, Pakrashi SC. The treatise of Indian Medicinal Plants. Publication and Information Directorate, New Delhi 1994; Vol.1: 1-180.
8) Arora R, Gupta D, Chawla R, Sagar R, Sharma AK, Parassad J, et al. Radioprotection by plant products: present status and future prospects. Phytother Res 2005; 19: 1-22.
9) Chawla R, Arora R, Kumar R, Sharma A, Prasad J, Singh S, et al: Antioxidant activity of fractionated extracts of rhizomes of high-altitude *Podophyllum hexandrum*: role in radioprotection. Mol Cell Biochem 2005; 273: 193-208.
10) Goel HC, Agrawala PK, Pathania V, Malhotra N. Immunomodulatory and cytoprotective role of RP-1 in γ-irriadiated mice. Molecular and cellular Biochemistry 2003; 254: 73-81.
11) Arora R, Sagar R, Singh S, Kumar R, Sharma AK, Prasad J, et al. Cytoprotective effect of *Podophyllum hexandrum* against γ radiation is mediated via hemopoietic system stimulation and up-regulation of heme-oxygenase-1 and the prosurvival multi-domain protein Bcl-2. Integrative Cancer Therapies 2007; 6(1): 54-65.
12) Gupta ML, Tyagi S, Flora SJS, Agrawala P, Choudhary P, Puri SC, et al. Protective efficacy of semi purified fraction of high altitude *Podophyllum hexandrum* rhizomes in lethally irradiated Swiss albino mice. Cellular and Molecular biology 2007; 53(5): 29-41.
13) Lata M, Prasad J, Singh S, Kumar R, Singh L, Chaudhary P, et al. Whole body protection against lethal ionizing radiation in mice by REC-2001: A semi purified fraction of *Podophyllum hexandrum*. Doi No. 10.1016/j.phymed. 2007. 04. 010.
14) Kumar R, Sing PK, Sharma A, Prasad J, Sagar R, Singh S, et al. *Podophyllum hexandrum* (Himalayan Mayapple) provides radioprotection by modulating the expression of proteins associated with apoptosis. Biotechnol appl Biochem 2005; 42:81-92.
15) Scott Luper ND. A review of plants used in the treatment of liver disease: Part I. Altern Med Rev 1998; 3(6): 410-421.
16) Singh M, Tiwari V, Jain A, Ghoshal S. Protective effect of picroliv on hepatic ameobiasis associated with carbon tetrachloride toxicity. Indian J Med Res 2004; 121: 676-682.
17) Sagar RK, Chawla R, Arora R, Singh S, Krishna B, Sharma R K, et al. Protection of the hemopoietic system by *Podophyllum hexandrum* against γ radiation-induced damage. Planta Med 2006; 72: 114-120.
18) Jagetia GC, Baliga MS, Venktesh P. Influence of seed extract of Syzygium cumini (jamun) on mice exposed to different doses of γ-radiation. J Radiat Res 2005; 46: 59-65.
19) Weiss JF, Landauer MR. Protection against ionizing radiation by antioxidant nutrients and phytochemicals. Toxicology 2003; 189: 1-20.
20) Shukla SK, Chaudhary P, Kumar IP, Samanta N, Afrin F, Gupta ML, et al. Protection from radiation induced mitochondrial and genomic DNA damage by an extract of Hippophae rhamnoides. Environmental and Molecular Mutagenesis 2006; 47: 647-656.
21) Goel HC, Prasad J, Singh S, Sagar RK, Agrawala PK, Bala M, et al. Radioprotective potential of a herbal extract of Tinospora cordifolia. J. radiat. Res. 2004; 45: 61-68.
22) Rios JL, Giner R M, Prieto JM. New findings on the bioactivity of lignans. Studies in Nat Prod 2002; 26 (Part G): 183-292.
23) Evans CR. Flavonoids and isoflavones: absorption, metabolism, and bioactivity. Free Radiat Biol Med 2004; 36: 827-828.
24) Khajuria A, Gupta M, Singh S, Malik F, Singh J, Suri KA, et al. RLJ-NE299A: A new plant based vaccine adjuvant. Phytother Res. 2006; 20(4): 316-321.