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BIOAVAILABILITY, ANTIOXIDANT AND NON TOXIC PROPERTIES OF A RADIOPROTECTIVE FORMULATION PREPARED FROM ISOLATED COMPOUNDS OF *Podophyllum hexandrum*: A STUDY IN MOUSE MODEL

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Abstract

The current study was aimed to determine the stability, serum protein binding ability, biodistribution, antioxidant potential and tissue toxicity status of a novel radioprotective formulation (G-002M) from *Podophyllum hexandrum*. G-002M is the combination of a flavonoid, a lignan and its glucoside isolated from *P. hexandrum* rhizome that exhibit high radioprotective potential. Stability of G-002M tagged with ^{99m}Tc was observed *in vitro* and with mice serum till 24 hr of incubation. The formulation was investigated for its antioxidant status and its bioavailability and toxicity in different organs of mice. Biodistribution study of ^{99m}Tc-G-002M revealed its uptake by all the vital organs of mice. Higher absorbed dose was observed in lungs, liver, jejunum and kidney. Maximum retention of G-002M in kidney revealed that G-002M was excreted predominantly through renal route. G-002M was also observed to have high free radical scavenging and total reducing properties. Histopathological observations showed no significant alterations in tissue morphology of lungs, liver, jejunum and kidney by G-002M administration. The data conclusively demonstrate that high stability, multi organ availability, longer retention and non-toxic behavior of G-002M might help in exhibiting strong protective potential against lethal radiation.

Key words: Ionizing radiation, radioprotector, *Podophyllum hexandrum*, biodistribution, flavonoid, lignan.

Article information

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INTRODUCTION

Ionization radiation has been implicated in causing injuries to normal tissue and is a key mediator for cell killing, altered cell to cell communication, inflammatory responses, and declined tissue repair processes (25, 26). To ameliorate radiation-induced morbidity and mortality, protection towards radiation exposure is inevitable. Radiation protection is a multifactorial phenomenon involving a number of mechanisms like immunostimulation, prevention of DNA damage, scavenging of free radicals, inhibition of lipid peroxidation etc. (10). Numerous synthetic compounds have been extensively evaluated for their radioprotective potential (1, 2, 9, 14) however, associated toxicity with effective concentrations has necessitated the search for radioprotectors that are potentially effective and minimally toxic (12).

Ionization radiation generates reactive oxygen species (ROS), which induces damage to critical cellular macromolecules (19). The endogenous defense mechanism is well proven in minimizing injurious effect of radicals generated during low radiation exposure, however, excessive production of ROS needs administration of exogenous supplements to suppress their deleterious effects. Compounds possessing the potential to neutralize the action of ROS might be promising in protecting cells from radiation exposure. Plant metabolites with various pharmacological properties (antioxidants, immunostimulants, antimicrobial etc.,) have attracted researchers to explore herbs for their therapeutic applications (29). However, most of the studies on plants have been on whole extract based preparations, which owe several limitations including ambiguity in understanding their mode of action. Therefore, it is necessary to identify the bioactive compounds and explore their mechanism of action in extending protective effects against radiation inflicted damages.

Out of many plants studied till date, *Podophyllum hexandrum*, Royale, family berberidaceae has been extensively investigated for its radioprotective potential. Previous studies with whole extract (6, 15, 21) and semipurified fraction (5, 13, 23) of *P. hexandrum* in animal models have revealed its potential to be used as a safe and effective radioprotector against lethal exposures. Most importantly, chemoprofiling data of these fractions revealed that *P. hexandrum* is rich in flavonoids and lignans, which are well documented to have high antioxidant potential (5, 8, 20, 27). Protection to peripheral blood lymphocytes, thymocytes, splenocytes and gastrointestinal epithelial cells by administration of *P. hexandrum* extracts have also been amply reported in pretreated and irradiated mice (6, 13, 21, 22). However, role of bioactive compounds of *P. hexandrum* in modulating lethal-radiation mediated damages either individually or in combination has not been revealed so far. Most of the studies conducted till date with *P. hexandrum* were mainly focused on whole body survival, toxicity and hematopoietic and biochemical parameters (5,

13).

Current study demonstrates the stability, biodistribution, antioxidant property and toxicity status of a novel formulation (G-002M) prepared by combining three compounds, belonging to lignan, lignan- glucoside and flavonoid group. Prophylactic administration of G-002M has been reported for rendering >90% whole body protection in mice exposed to lethal radiation (7). To understand the mode of action of G-002M in mediating protection against radiation exposure, *in vitro* and serum stability of ^{99m}Tc-G-002M and its binding ability with serum protein was studied. Biodistribution of ^{99m}Tc-G-002M was examined in mice. Organs showing high bioavailability of G-002M were processed for histological observations. The free radical scavenging and reducing properties of G-002M were also studied to assess its potential in minimizing deleterious effects of lethal radiation.

MATERIALS AND METHODS

Collection of plant material and extraction of G-002M

P. hexandrum grown under natural conditions at Pulwama, Jammu and Kashmir, India was authenticated by plant taxonomist from the Centre of Plant Taxonomy, University of Kashmir, Srinagar. A voucher specimen was deposited in the repository of IIIM, Srinagar (voucher no. RRL/PH/Srinagar-2005). The freshly collected rhizomes were shade dried in specially designed dust free chambers and all the precautions were taken to keep the material free from soil, dust and pathogens. Dried material was crushed to obtain homogeneous fine powder. The dried powder was extracted with petroleum ether. The marc was dried to remove residual solvent and then fractionated with methanol (four washes) using standard procedure.

For isolation of pure compounds, the above prepared extract was dissolved in water and the solution was successively extracted with chloroform, ethanol and n-butanol. The isolated lignans/flavonoids were identified and their purity was checked with the help of HPLC, HPTLC, IR, NMR and MS data using suitable standards (7).

HPLC analysis

The fractionated samples were analyzed on a Shimadzu LC-10 AT VP HPLC machine isocratically utilizing E-Merck RP-18 column (250 x 4.0mm, 5µm) with diode array fluorescent detector (SPDM-10A VP/RF-10 AXL) and autoinjector (SIL-10 AD VP). Elution of lignans was carried out at wavelength of 240 nm with mobile phase (Methanol:water) at a flow rate of 0.6 ml min^{-1}, while elution of flavonoid was carried out at 340 nm with mobile phase (0.05% Trifluro acetic acid in acetonitrile:0.05% Trifluro acetic acid in water) at a flow rate of 1ml min^{-1}. Ten microliter of the stock solution (200µg/ml) was injected separately into the column and samples were run for 70min. All the measurements were carried out while maintaining the column temperature at 29±2°C. Peaks were matched with respective standards (Merck/Sigma).

G-002M formulation

The G-002M formulation used in the present study is a combination of three active principles, belonging to lignan (A), lignan glucoside (B) and flavonoid (C) isolated from the rhizome of *P. hexandrum* and mixed in 3:1:1 ratio respectively. All the three active principles used for the study were in >95% purity as measured by HPLC and LC-MS methods.

Labeling of G-002M with ^{99m}Tc

The labeling method was initially performed with individual compound A, B and C. Each compound was dissolved in 10% DMSO and mixed with 50 µg of stannous chloride and the pH of the reaction mixture was adjusted at 7.5 with 0.1 N NaOH. To the reaction mixture 1.0 mci of (^{99m}Tc) TcO_4Na was added and incubated at different time intervals. Similar method was followed to label G-002M formulation containing all the three active principles. The radiochemical purity was analyzed using instant thin layer chromatography (ITLC) method.

The labeled reaction mixture (5 µl) was spotted 1.5 cm from the bottom of the ITLC strip (1.5 X 15 cm) and developed in acetone to detect free pertechnetate until the solvent reached the top of the strip. The ITLC strip was dried and cut into 0.5 cm sections. Radioactivity was measured with NaI (TI) detector (LKB Wallac Compu Gamma Counter) to determine the labeling efficiency and thus radiochemical purity of the complex. Free technetium was separated from ^{m99}Tc- labeled complex and moved ahead, whereas the labeled conjugates remained at the start line. The activity corresponding to the base of the strip compared to the total activity of the strip as 100% provided the radiochemical purity. All measurements were carried out under the same geometric conditions and in triplicate.

The percent colloid present in the ^{99m}Tc-labeled formulation was determined by ITLC method using pyridine-acetic acid-water (PAW; 5:3:1.5) as mobile phase.

Serum stability

G-002M was labeled with ^{m99}Tc and its stability with mice serum was evaluated up to 24 hr. Serum was isolated from mice blood collected by heart puncture. The percentage ^{m99}Tc activity dissociated from the formulation was assessed at different time intervals (15min, 1hr, 2hr, 5hr and 24hr) using ITLC method as mentioned before.

Protein binding estimation

Tricholoroactetic acid (TCA) precipitation method (11, 28) was used to determine the percentage of ^{m99}Tc-labeled formulation bound to total mice serum protein.

In Vitro Assay

Free radical scavenging activity

The free radical scavenging activity of all the three compounds individually (A, B and C) and as well in combination (G-002M) was performed as described by (16). The assay was based on the ability of compounds to stabilize 1,1-Diphenyl-2-picryl hydrazyl (DPPH) radicals and the scavenging activity was compared with standard antioxidants, Quercetin and BHT present in 98-99% pure form (Sigma Chemical Co., St. Louis, USA). Briefly, 1 ml of varying concentration (0.5–500 µg/ml) of compounds A, B, C, G-002M, quercetin and BHT were added to 1mL of 0.1mM DPPH in methanol. The reaction mixtures were incubated in the dark for 30 minutes, and the resultant purple color was measured at 517 nm against the standard.

Total reducing power

Reducing power of G-002M was determined according to the method of (17). Briefly, various concentrations of

compounds A, B and C individually and G-002M were mixed with 200µl 0.2M phosphate buffer (pH-6.5) and 0.1% potassium ferricyanide and incubated at 50°C for 20min. To the reaction mixture, 250 µl 10% TCA was added and centrifuged at 6000rpm for 10min. The supernatant was mixed with 500µl distilled water and 100µl 0.1% ferric chloride and incubated at 30°C for 10min. The antioxidant BHT was taken as standard to compare the reducing power ability. Absorbance was recorded at 700nm.

Animal studies

Strain 'A' female mice (6-8 weeks old; weighing 26±2g) were selected for the study. Animal experiments were performed in accordance with the Institute's animal ethical committee guidelines (approval no. INM/IAEC/2010/07/04 dated 09.06.2010). The mice were maintained in well ventilated, temperature controlled (25±2ºC) animal room and provided food and water *ad libitum*.

Biodistribution in mice

For biodistribution studies, each animal (n=3 for each time point) was injected with 200µL of the ^{m99}Tc labeled G-002M (2µg/µl) intramuscularly. The animals were sacrificed at different time intervals (15min, 30min, 1hr, 2hr, 5hr and 24hr) after administration of the formulation. Blood and organs of interest (spleen, thymus, lungs, liver, bone marrow, kidney, whole brain, jejunum and stomach) were removed and weighed. Total radioactivity was counted in whole blood and other organ tissue with γ counter. The percentage dose per gram of tissue was calculated by comparing the sample counts with count of the diluted initial dose.

Histological evaluation

Female Strain 'A' mice from control and G-002M treated groups were dissected 72hr post treatment. Liver, kidney, lungs and jejunum were excised and fixed in 10% buffered formalin. 5µm thick sections were prepared from tissue embedded paraffin blocks and stained with hematoxylin and eosin using standard protocol. Changes in tissue organization of organs fixed from various groups were carefully recorded. At least three separate sections of each organ from each treatment group were studied.

Statistical analysis

All experimental data are expressed as mean ± SD.

RESULTS

Chemoprofiling of the rhizome extract of Podophyllum hexandrum

Chemoprofiling of the rhizome extract of *Podophyllum hexandrum* revealed the presence of various secondary metabolites. Beside many active principles, podophyllotoxin and podophyllotoxin-β-D-glucoside were identified as major lignans (Fig.1). Similarly, HPLC analysis also confirmed the presence of a variety of flavonoids, including, quercetin, quercitrin, rutin and kaempferol (8). The chromatogram of flavonoid used in the study is represented in figure 2. Based on the chemoprofiling data, one lignan, one lignan - glucoside and a flavonoid were selected for preparation of G-002M formulation.

The antioxidant property of individual active principles and in combination (G-002M) was evaluated in terms of free radical scavenging and total reducing activities.

Free radical scavenging activity

The free radical scavenging activity was measured by their ability to scavenge DPPH radical. Figure 3 shows the dose-response curve of DPPH radical scavenging activity of compounds A, B, C and G-002M, compared with standards BHT and quercetin. Compound A and B were found to have low scavenging activity (10% and 20% respectively), and the % scavenging was independent of the concentrations tested. DPPH scavenging activity of compound C and G-002M was observed to be concentration dependent. At a concentration of 0.5ug/ml, the DPPH scavenging activity of compound C was 30%, while at 10ug/ml the scavenging activity was 80%. At 20ug/ml concentration the scavenging activity was found to be saturated. The radical scavenging activity of G-002M was found to be higher than standard BHT but equivalent to quercetin.

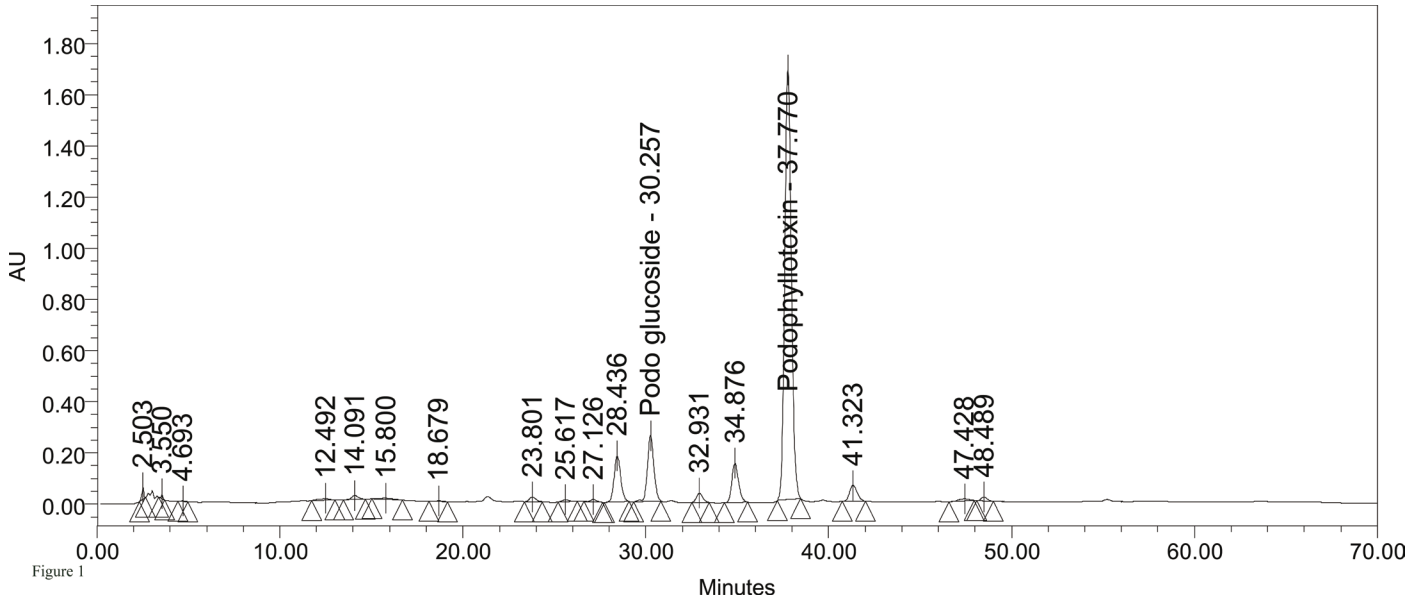

Figure 1. HPLC chromatogram of methanolic extract of *Podophyllum hexandrum* showing presence of various secondary metabolites. Podophyllotoxin and podophyllotoxin-β-D-glucoside were identified as major peaks in the chromatogram.

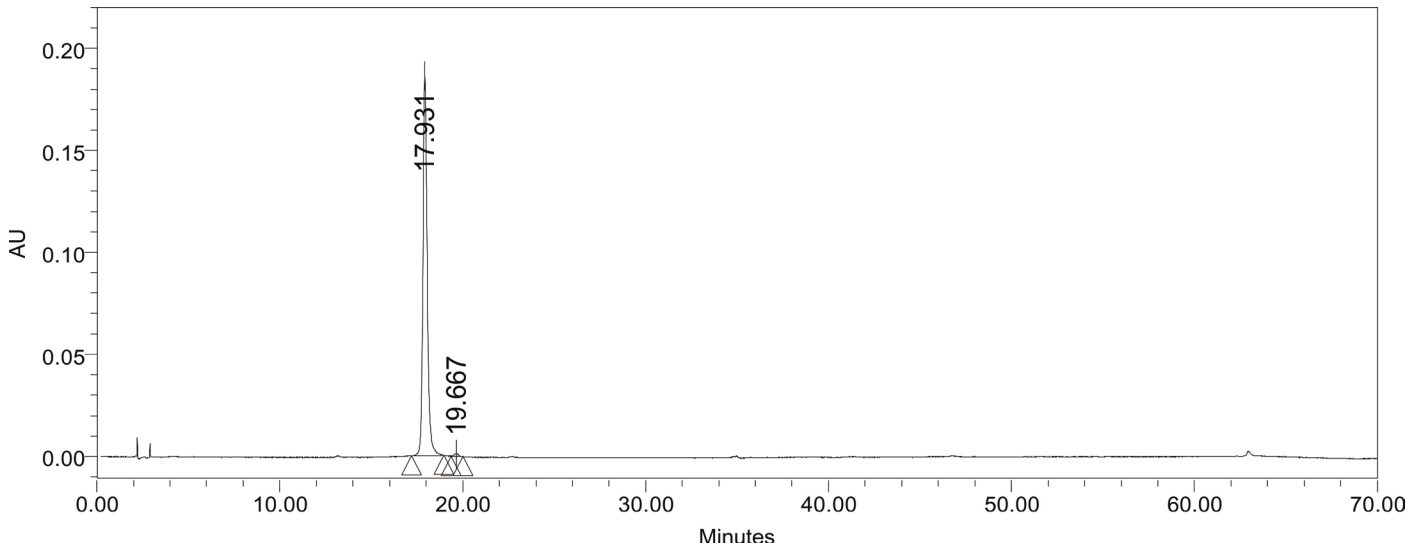

Figure 2. HPLC chromatogram showing presence of single peak of flavonoid used in the study.

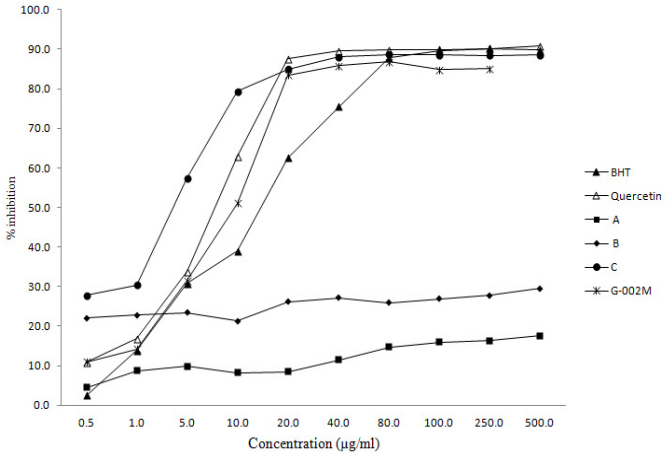

Figure 3. 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of different concentrations of compounds A, B, C and G-002M. The % inhibition of free radicals by compounds A, B, C and G-002M are compared with reference standards BHT and Quercetin. Results are expressed as mean ± S.E. of three parallel measurements.

The % scavenging activity of compound C was higher than both the standards (BHT and quercetin; Fig. 3).

Total reducing power

The reducing power of compounds A, B, C and G-002M and their comparison with standard BHT is illustrated in fig. 4. The reducing ability of compounds A and B were found to be almost similar at 2ug/ml, while reducing ability of compound C and G-002M was observed 8-9 fold higher at similar concentration. Comparison with the standard BHT revealed higher reducing power of compound C compared to A and B (Fig. 4).

Furthermore, studies were carried out to elucidate the *in vitro* and serum stability, serum protein binding, distribution, retention and excretion of G-002M formulation in mice.

In vitro labeling of ^{99m}Tc-labeled G-002M

Initially the coupling of ^{99m}Tc radiotracer to A, B and C were tested individually. Incubation of each compound with ^{99m}Tc for 15 min provided > 97% yield at room temperature. High stability was maintained for ^{99m}Tc labeled compound A and B till 24 hr. Compound C revealed nearly 98% yield till 1 hr of labeling, however the yield was reduced to 91% by 24 hr (Fig. 5). Under similar condition, G-002M was labeled with ^{99m}Tc (^{99m}Tc-G-002M) and checked for the stability till 24 hr. More than 99% yield was attained after 15min of incubation with ^{99m}Tc and the high yield was maintained till 24hr. Storage of the labeled formulation both individually or in combination in 10% DMSO up to 24 hr did not cause any significant release of ^{99m}Tc radioligand, suggesting high stability of the complex (Fig. 5). No significant colloids (<2%) were formed during the labeling of G-002M with ^{99m}Tc.

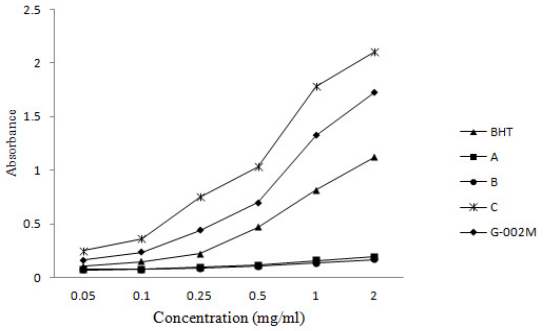

Figure 4. Reducing power of compounds A, B, C and G-002M. The reducing ability of different concentrations of individual compound was standard BHT. Absorbance is measured at 700nm against blank and results are expressed as mean ± S.E. of three parallel measurements.

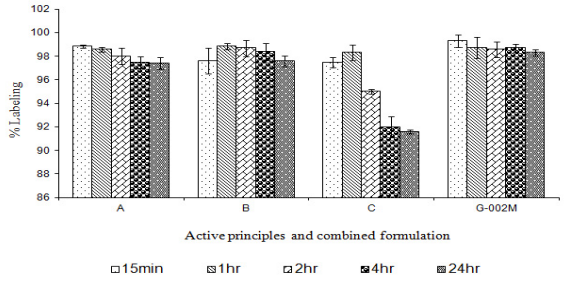

Figure 5. Determination of labeling yield for ^{m99}Tc-G-002M and its individual component at different time intervals. The percentage ^{m99}Tc activity dissociated from compounds A, B, C and G-002M was assessed as function of time. Labelling yields are presented as mean from of three independent experiments ± S.E.

Serum stability of the ^{99m}Tc-G-002M complex

The stability of ^{99m}Tc-G-002M complex with mice serum was assessed over a 24hr time period. The serum stability data revealed that nearly 99% yield was attained within 30min of incubation and the complex was found to be very stable up to 24hr at the conditions tested. No significant dissociation of the radioligand from G-002M-serum complex was observed. The percentage of ^{99m}Tc released from this complex at 24hr was < 3% (Fig. 6).

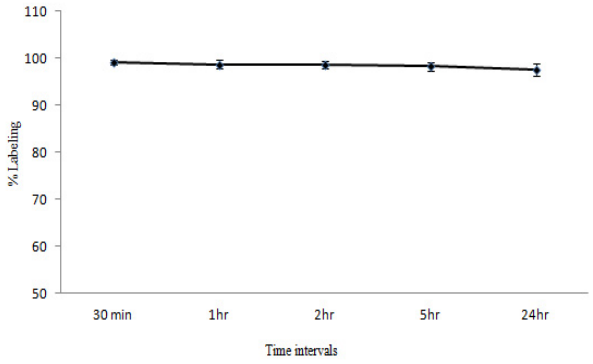

Figure 6. Serum stability study of ^{99m}Tc-G-002M complex at various time intervals upto 24hr. The percent of bound ^{99m}Tc-activity to G-002M complex is plotted as a function of time. Each data point represents the

Binding of serum protein to ^{99m}Tc-G-002M complex

We investigated the binding of ^{99m}Tc-G-002M to the mice serum protein at 30min, 1hr and 2hr incubation time, which indicated high affinity of G-002M to serum protein. The results demonstrated that nearly 61.6%±1.1 of G-002M was bound to serum protein within 30min. The percentage of bound proteins after 1hr and 2hr was found to be 74.02% ± 1.9 and 84.47%±2.2 respectively. These results demonstrated a strong conjugation between serum proteins and G-002M, which might further facilitate in the distribution of G-002M to different organs.

Biodistribution of ^{m99}Tc- G-002M in mice

^{99m}Tc-G-002M showing more than 98% yield was injected in mice by intramuscular route for biodistribution study. The data on biodistribution of the ^{99m}Tc-G-002M is summarized in table 1. Availability of G-002M in mice was observed within 30 min post administration in blood, bone marrow and major organs (spleen, thymus, liver, kidney, jejunum and stomach), although at different levels. Maximum retention of G-002M was observed at 2 hr in most of the organs. The absorbed dose was reduced by 5hr of administration and was completely eliminated by 24hr of administration. At all time points, higher retention of ^{99m}Tc-G-002M was evident in thymus, lungs, liver and kidney. Higher retention in kidney of radiolabeled G-002M suggested excretion of G-002M majorly through the renal route and to a lesser extent through hepatobilary route.

Since, higher retention of G-002M was evident in lungs, liver, kidney and jejunum, so we investigated the pathologies of these organs for any morphological alterations after 72hr of administration of G-002M.

Histology of mice tissues

Liver section showed presence of portal triad and central vein surrounded by hepatocytes with well defined nuclei and cytoplasm similar to controls (Fig. 7a). No change in the liver morphology including fatty liver changes was evident after 3 days of G-002M treatment. G-002M treated tissues were in perfect correlation with untreated mice liver (Fig. 7b).

The lung tissue architecture of control mice was found to have terminal bronchioles, veins, alveoli ducts and alveoli sacs (Fig. 7c). Three days post G-002M treatment no accumulation of edematous fluid, deformation of bronchial epithelium and alveoli in any of the histological preparations were evident. Bronchioles were also found to be normal (Fig. 7d). Overall the lungs pathology in G-002M treated group was similar to the untreated group.

Comparison of kidney section from G-002M treated animals with the control showed no significant change in

Table 1. *In Vivo* biodistribution of m99Tc -G-002M complex in control Strain 'A' mice.

Organ	15min	30min	1hr	2hr	5hr	24hr
Blood	0.92±0.16	1.00±0.05	0.77± 0.07	0.66±0.09	0.57±0.05	0.58±0.15
Bone Marrow	0.07±0.02	0.05±0.01	0.04± 0.002	0.03±0.005	0.02±0.01	0.008±0.002
Spleen	0.16±0.02	0.27±0.05	0.30±0.05	0.41±0.1	0.28±0.03	0.23±0.04
Thymus	0.3±0.05	0.76±0.02	0.91±0.12	1.11±0.60	1.36±0.18	0.77±0.07
Lungs	0.51±0.12	0.81±0.17	0.85±0.01	1.44±0.91	0.62±0.08	0.46±0.04
Liver	0.80±0.15	0.98±0.09	1.31±0.13	1.44±0.29	2.12±0.27	1.25±0.15
Whole Brain	0.04±0.003	0.04± 0.008	0.04±0.005	0.04±0.004	0.03±0.005	0.02±0.006
Kidney	1.03±0.2	1.47±0.14	2.7±0.21	5.12±0.56	4.27±0.84	4.47±0.75
Jejunum	0.62±0.17	0.44± 0.05	0.53± 0.06	0.56±0.02	0.70±0.15	0.60±0.17
Stomach	0.85±0.13	0.25±0.06	0.29±0.01	0.20±0.05	0.28±0.01	0.30±0.07

Each animal (n=3 for each time point) was injected with 200µL of the ^{m99}Tc-G-002M (2µg/µL) intramuscularly. The animals were sacrificed at different time intervals. Organs of interest (spleen, thymus, lungs, liver, bone marrow, kidney, jejunum and stomach) were removed and weighed, and the radioactivity was counted with γ counter. Data are % dose/g, average of 3 mice± SE.

tubular epithelium and glomeruli architecture. The renal cortex, stroma and medular regions were found unaltered after G-002M pretreatment (Fig. 7e-f). Cells were found with perfect shape and size with normal nucleus and cytoplasm.

Jejunum cross sections were also checked for atrophy, edema, necrosis and clubbing in crypt, villi, lamina propria and muscular layers. Villi shape and length did not altered due to G-002M treatment. Mitotic index (4-6%/crypt) and apoptotic cells/crypt (0.05-0.2 cells/crypt) were more or less same as in untreated group. Crypts comprised of abundant dividing cells. No significant changes in the morphology of jejunum tissue was evident in G-002M treated animal when compared with the control tissue (Fig. 7g-h).

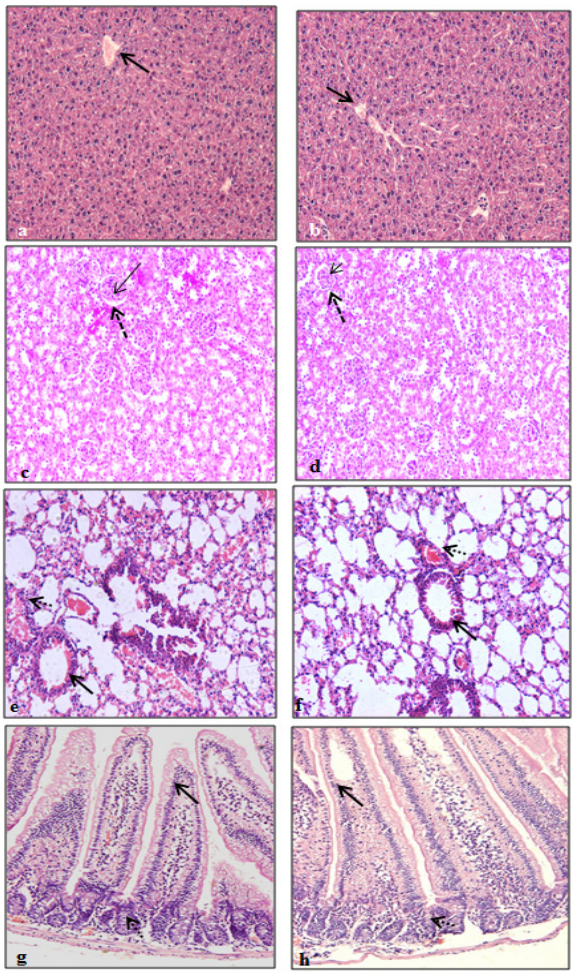

Figure 7. Photomicrography of liver, kidney, lungs and jejunum tissues of control and G-002M treated mice. Strain A mice were injected intramuscularly with G-002M and tissues were excised 3 days after treatment. Tissues were processed for histological evaluation and were assessed for any alterations in structural organizations.

a-b: Cross section of control (a) and G-002M treated (b) liver with normal hepatic venule (arrows), hepatocytes and sinusoidal spaces. X100.

c-d: Cross section of control (c) and G-002M treated (d) kidney. Bowman's capsule containing glomerulus (arrow) and Bowman's spaces (dotted arrows) are seen. X100.

e-f: Cross section of control (e) and G-002M treated (f) lung with normal terminal bronchiole (arrows), veins (dotted arrows), alveolar ducts and alveolar sacs. X100.

g-h Morphology of jejunum of Strain A mice either untreated (g) or 3 days after G-002M treatment (h). Mucosal layer with compact epithelial cells lining of villi (arrows) and cypts (dotted arrows) are seen in the histological preparation. Lacteals and muscle layers were observed normal. X100.

DISCUSSION

Studies conducted in last one decade in our laboratory have revealed great potential of *P. hexandrum* in modulating lethal radiation mediated cellular injuries (13, 21, 22). Chemoprofiling of *P. hexandrum* rhizome extracts has conclusively demonstrated that this plant is a rich source of several flavonoids like quercetin, quercitrin, rutin and kaempferol and lignans like podophyllotoxin, podophyllotoxin glucoside, demethyl podophyllotoxin. Flavonoids are well documented to exhibit strong antioxidant activities, which may be attributed to their direct hydroxyl radical scavenging potency (24). On the other hand, lignans are reported to be potential immunomodulators and DNA protector (30). Intramuscular administration of single dose of G-002M in Strain 'A' mice one hour prior to exposure to lethal radiation rendered > 90% whole body protection (7). Better survival results were obtained with pre-administration of G-002M compared to its crude and semipurified preparations in mouse model (7).

Irradiation of a living cell leads to a rapid burst of ROS and severity of damage depends on the balance between the rate of ROS production and the rate of its elimination by endogenous and exogenous interventions (4). Polyphenols are the major plant metabolites known to have high antioxidant activity. The antioxidant potential of polyphenol is thought to be mainly due to its role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen and decomposing peroxides predominantly by donating hydrogen atom from their associated hydroxyl groups (4). Though the reducing power and free radical scavenging ability of compounds A, B, C and G-002M were found to be variable, the study showed that compound C have comparatively high proton donating ability and could serve primarily as free radical scavenger. Our formulation, G-002M is a combination of flavonoid, lignan and lignan glucoside and its radioprotective potential may be attributed partially to the strong redox property of flavonoid used in G-002M formulation. Our data clearly indicate the protective mechanism of the cells from excessive proton production, which in turn contributes to restoration of antioxidant balance disturbed by ionization radiation. Lignan, a major contributor of G-002M has also been reported as cell cycle arrester at G2-M phase which facilitate the cells to undergo repair of DNA related damages. Lignan glucoside, another component of G-002M is well documented as immunomodulator. All the three compounds probably have worked in synergy to provide protection against radiation.

Stability of radioprotective formulation is of crucial interest because the formulation must stay in the tissues for longer period to protect the sensitive organs at the time of irradiation and also extend support to maintain the functionality of broad range of cellular and tissue systems. ^{99m}Tc radionucleide was selected for stability and biodistribution studies because of its frequent use in nuclear diagnostic technology with almost zero toxicity, near ideal nuclear characteristics of short biological half life and γ-ray emission energy of 141 keV (3). Our stability data was very promising as more than 95% ^{99m}Tc was found bound with G-002M till 24hr. A stable radiolabeled G-002M was obtained as shown *in vitro* and with mice serum by the absence of decomposed products in particular pertechnetate, after incubation for 24hr. The interaction of different drugs

with circulatory proteins is an important property influencing the distribution of the drug and its elimination from the body. In the present study, we observed high degree of association of ^{99m}Tc-G-002M to total serum protein. Our observation corroborated with the finding of Rassmann et al., (18) suggesting high percentage of protein binding of NK 611, a podophyllotoxin derivative. The radiolabeled G-002M was prepared with a high specificity, allowing to quantitate its binding to serum proteins and to determine its biodistribution in mice. In mice, Tc^{99m}-G-002M was distributed primarily to blood, lungs, liver and kidney and had high degree of serum protein binding. Strong binding might have allowed the formulation to reach specific target tissues such as blood, lungs, liver, jejunum and kidney and same was observed in biodistribution studies in mice. Retention of G-002M till five hour post intramuscular administration to mice and wide distribution of this formulation to various organs has helped in saving the organs against free radical attack and also minimized further propagation of radical formation. In addition, presence of our formulation prior to irradiation has also facilitated in enhancing internal defense mechanism.

Tissue histology studies showed that G-002M administration caused no detrimental effect on the structural organization of kidney, liver, lungs and jejunum. The dose of G-002M used in the present study was selected from the previous report (7), which showed that the tested dose is sufficient to provide protection against lethal radiation inflicted damages and also safe in use at its effective dose.

Extensive research conducted till date has failed to develop a radioprotector that fulfills all the desired criteria of potential radioprotector. Studies based on singular compound of either synthetic or herbal origin so far have not yielded the significant results. Based on our detailed studies we believe the active principles present in the G-002M are possibly acting in synergy as free radical scavenger, immunomodulator and DNA and other cellular macromolecule savor and thus conferring whole body protection to mice against lethal radiation.

In conclusion, the formulation (G-002M) from *P. hexandrum*, reported earlier for > 90% whole body protection against lethal radiation (7) distributes to almost all the potential organs of the body and its retention for sufficiently larger duration might be helpful in providing protection against lethal radiation exposure. In conjunction with the findings on the G-002M, it can be concluded that this formulation of *P. hexandrum* fulfills several criteria of a safe and effective radioprotective formulation.

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