Radio protective herbal formulation against UV exposure

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Abstract Exposure to high amounts of ionizing radiation or other radiations causes damages to the hematopoietic, gastrointestinal or central nervous systems. Due to the damage caused by ionizing radiation and its impact on the human life, there is a need to develop an effective and non-toxic radioprotectent. Various herbs has been mentioned as radioprotectant in the Ayurvedic treatment with negligible side effects. Some of the Herbal extracts with therapeutic benefit may work as a potential radioprotectent in intentional as well accidental radiation. This study aims at finding out the efficacy of organic Berberine and Selenium as possible radioprotectant. Experiment has been performed on E coli DH5α at different durations (at 15, 30 and 60 minutes) of radiation (UVC radiation from 100nm-280nm) and at various concentrations of Berberine (drug D1) and Bereberine+Selenium (drug D2) treatment. Analysis of DH5α was done by spread plate method, colony counting after drug D1 and D2 treatment, DNA damage and protein expression profile with and without UV irradiation. Biochemical assays were also performed to asses GST, GSH and CAT activity. Results after 30 minutes of UV exposure with drug D1 and D2 treatment were analysed. It showed that 20µg/ml of drug D1 has radioprotective activity as per spread plate method, however drug D2 at the concentration of 40µg/ml has shown the colony enhancement as well as DNA protectant ability by keeping the DNA intact on the gel as compared to the UV exposed control plate, which has shown DNA shearing after UV exposure. CAT was found to be highest in drug D2 at the concentration of 40µg/ml i.e. 0.7umol/min/ml after 30 minutes of exposure. GST and GSH were also found highest in drug D2 which was in the concentration of 20ug/ml i.e. 0.021µmol/min/ml and at the concentration of 60µg/ml which was 0 .0185µmol/min/ml respectively. Later a cream based formulation of the drug D1 and D2 was prepared using 2:1 ratio of the drugs. Formulation stability was assessed by Organoleptic evaluation. It has been observed through the experiment that drug D2 at 40µg/ml in the ratio of 2:1 (Berberine+selenium) was able to provide necessary protection against UV radiation.

KEY WORDS: Radio protective agents, free radical scavenging activity, herbal radioprotectors, ionising radiation,antioxidant.

I. Introduction

Radiation is indeed a part of our daily life but its exposure to the biological system could be lethal and may produce deleterious effects such as DNA damage, protein damage and lipids peroxidation on human body.The unexpected alternations or damages in biological systems can take place through intentional and accidental radiation exposures. There have also been incidents of genetic defects in the offsprings, whose parents were exposed severely to the radiations. DNA damage can result in alteration in the encoded proteins, which later on may lead to complete inactivation or malfunctions of the encoded proteins (Bala et al., 2014).

There are various types of radiations and most of them are undetected to the humans. Both ionizing and non-ionizing radiation could be harmful to living organisms but exposure to high amount of ionizing radiation can cause damage to the vital organs and can suppress immune system, which later on results in damage to the cells in the living organisms (Yamini and Gopal, 2010). Exposure to the radiation also causes ionizations in the molecules of living cells which results in formation of free radicals, reactive oxygen species (ROS) and reactive nitrogen species (RNS) which react with other molecules in the cells & cause damage in the cells (Beal, 2002).

The deleterious effect of UV radiation (UVR) is highly dependent on the wavelength of radiation. DNA is the major chromophore following exposure to short-wavelength UVR. Both ultraviolet C (UVC) (<290 nm) and ultraviolet B (UVB) (290 to 320 nm) can induce the formation of cyclobutyl pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidinone photoproducts, which are mutagenic and lethal to bacteria if unrepaired (Pfeifer, 1997; Qiu et al., 2005). Damage induced by long-wavelength UVR is more complex, since a variety of non-DNA targets with a maximum λ in the range of 290 to 400 nm are present in the cell (Eisenstark, 1987 & 1989).

There are various repair activities which is evolved in our system in order to repair damaged bio-molecules and protect cells at low levels of damage but the most vulnerable level is nuclear DNA damage, which may not be repaired in most cases. It has been observed by many scientist that many bacteria has evolved various mechanisms to cope with UVR-induced damage. In Escherichia coli, both photo reactivation and nucleotide

excision repair (NER) are highly efficient in removing CPDs (cyclobutyl pyrimidine dimmers) (Kim and Sancar, 1993; Sancar, 1996), whereas recA-mediated recombination repair can bypass CPDs during DNA replication, thus improving DNA damage tolerance (Friedberg et al., 1995; Miller and Kokjohn, 1990). The LexA-RecA mediated SOS response is a global response to DNA damage involving the induction of more than 30 unlinked genes, many of which are involved in DNA replication and repair and in the control of cell division (Courcelle et al., 2001; Little and mount, 1982).

Radioprotectors are compounds that are designed to reduce the damage in normal tissues caused by radiation. Radioprotectants from natural sources will have great value because they are easily obtainable, economical and non-toxic. Natural radioprotectors make use of their protective actions by quite a few mechanisms such as: 1) Scavenging of free radicals or antioxidant mechanisms (Zhao Y et al. 1995), 2) Up regulation of mRNAs of antioxidant enzymes like CAT, GST, GSH, GPX, SOD (Neta R 1988), 3) Support the recovery of hematopoietic and immune functions (Chiu SM. et al., 1998), 4) Condensation of DNA (Venkatachalan SR. et al., 2005), 5) Activation of the DNA repair enzymes (Chiu SM. et al., 1998), 6) Detoxification of the radiation stimulated reactive species (Vijalakshmi et al., 1998), 7) By delay in the cellular division and by inducing hypoxia in the tissues (Nair CKK. et al., 2001), 8) Also Reduction in lipid peroxidation and increase in non-protein sulfhydryl groups (Devasagayam et al., 1996), 9) Immuno modulation and Facilitation of repopulation of damaged & affected organs is the other way of detoxification (Yamini, K. and Gopal, V.,2010). 10) Slowing down the activation of protein kinase, nitrogen trigger protein kinase, cytochrome P-450, nitric oxides etc has been also reported as an efficient way of minimizing radiation effect (Jagetia et al. 2004). Plants extracts contains a vast array of compounds including antioxidants, cell proliferators, stimulators, immune modifiers and anti-microbial activities other than anti-radiation activity (Arora et al., 2005; Jagetia et al., 2002; and Maurya et al., 2006). So, Recent researches are showing their interest on herbal plants and their products for human benefits.

Natural compounds with antioxidant activity have potential as good radioprotectors besides, Selenium and vitamin-E has been reported as good radioprotectors (Bakir et al., 2005; Devasagayam et al., 2006). Selenium has been shown to be a direct free radical scavenger and an indirect antioxidant (Sood et al., 2011). Moreover selenium might play a role in the prevention of cancer induced due to UV radiation. Another herbal compound-Berberine is found to be a natural anti-inflammatory agent and various researchers have also performed test with its extract and found it to be effective in reducing the severity of radiation-induced intestinal syndrome in many patients undergone radiation therapy to treat various cancers. (Rahul et al., 2014)

In the current study, efficacy of the berberine and selenium was checked against UV radiation on E. coli DH5α as herbal radioprotectant. The rationale of selection of E. coli DH5α was that, it has been used as efficient transforming and expression agent by various scientists across the globe.

Material and Method

Sample Collection:

Pure compound Berberine and organic selenium were procured from sigma. E. coli strain DH5α by medox was procured from New Delhi. The strain is quite popular amongst scientists for its transformation efficiency due to the presence of two mutated genes EndA1 and recA, and having a very stable plasmid DNA. E. coli DH5α was revived in LB (Luria broth) and plated on the LA (Luria agar).

Colony Morphology Study:

Experiment was divided into 4 groups. 1st group constituted negative control (unexposed to UV). 2nd group constituted three plates (positive control in triplicate) exposed to UV for consecutive 15min, 30min, and 60min respectively. 3rd group constituted of UV exposed plate for 30 minutes, (all four plate) carrying 20µg/ml, 40µg/ml, 60µg/ml and 80µg/ml berberine (drug D1) in the media. To check the efficacy of the compound, 4th group had berberin+selenium (drug D2) (in the ratio of 2:1) 20µg/ml, 40µg/ml, 60µg/ml in the media sonicated at 0.5 cycles, 40% amplitude at room temperature then exposed to UV for 30 minute each.

DNA Damage and Protein Expression Analysis:

DNA was isolated by phenol-chloroform method and 1% Agarose gel was prepared to separate DNA from all samples (Sambrook et al., 1989). Protein estimation was done by urea lysis method and quantified by Bradford method. Later protein was separated by SDS-PAGE to visualize the up regulation and down regulation of the antioxidant proteins (Sambrook and Rusell, 2006).

Biochemical Assay of the treated and exposed bacteria:

Biochemical analysis was done by catalase in which the color change using spectrophotometer was observed at 240 nm (Sinha, 1972), GST (Glutathione s-transferase), its optical density was taken at 340 nm wavelength (Habig et al., 1974). And Optical density for GSH (Glutathione sulphur Reductase) was observed at 412nm (Owens and Belcher, 1965).

Formulation of the natural radio protectant cream:

Later the most effective concentration was taken to form gel based cream using Berberine and Selenium (drug D2) in 2:1 ratio. The technical feasibility of the formulation was done depending upon the various forms such as no variation in physical – chemical properties, organoleptic character, and no contamination on keeping parameters. Stability of the cream and its organoleptic examination was done as per Grace and Vijetha (2014).

Results

Colony Morphology Study:

Figure1 depicts the effect of UV exposure for various durations on E. coli DH5α colony with the protection provided to the organism through drug (D1 and D2) treatment at different concentrations. It has been observed that the maximum damage was done at 60 minute of UV exposure as compared to the 15 minutes exposure to non-treated DH5α. Drug D1 was potent in providing the UV protection at the conc 20µg/ml, however drug D2 was found to provide protection at the conc of 40µg/ml.

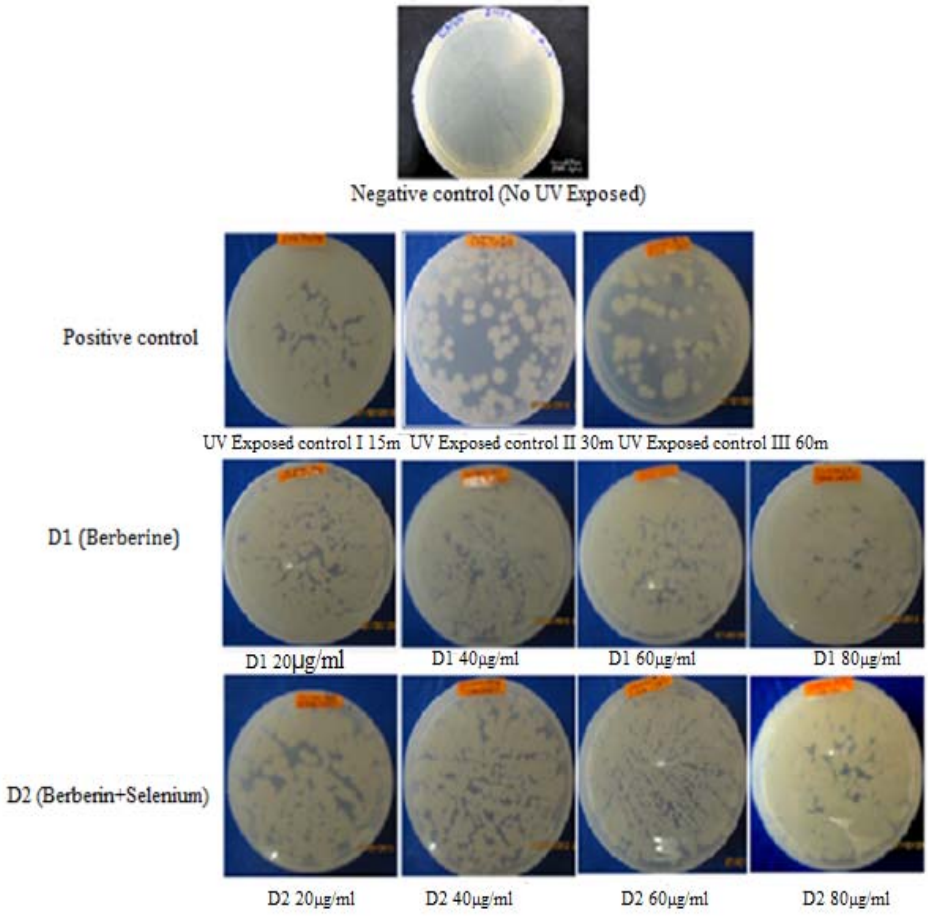


Figure1: Shows the comparative account of the UV exposure on E. coli DH5α with Drug D1 and D2 along with positive and negative control.

DNA Damage Analysis:

DNA was isolated and expression of the DNA of treated (with drug D1 and D2) and UV exposed DH5α is compared with control (UV exposed positive and Non-UV exposed negative control) was observed after 30 min. It has been observed that DNA was found seveared in UV exposed untreated plates, however DNA of the plates treated with D1 and D2 drug has been found to be intact. Drug D1 at the concentration of 20µg/ml and 40µg/ml and Drug D2 at the concentration of 40µg/ml & 60µg/ml showed protection against UV radiation.

Figure 2: Gel picture showing negative (lane 3) and positive (lane 2, 8 & 9) control with UV exposed after 30 minutes and with treatment of different concentration of D1 (lane 4&5) and D2 (lane 6&7) Drugs.

Protein Expression Profiling:

The main focus was on antioxidant protein which might get up regulated or down regulated due to the treatment with D1 and D2 drug. In this expression the protein which is approximately of 96kDa was found to be down regulated. The protein in the range of 66kDa- 97 kDa was found to be up regulated after treatment with drug D1 at concentration of 20µg/ml and 60µg/ml & in drug D2, it was found at the concentrstion of 20µg/ml & 40µg/ml. It could be the surface protein such as HSP70 as it lies in the same range. It's up regulation usually means that the organism is gearing up for protection against uv radiation. Interestingly, proteins in 43- 66 kDa were found to be upregulated which can be (Melanocortin 1 receptor protein) MC1 Receptor or MSH (melanocyte stimulating hormone for alpha, beta & gamma), and (Adrenocorticotropic hormone) ACTH. The activities of these proteins were found to be mediated by G protein which further activates adenylatecyclase.

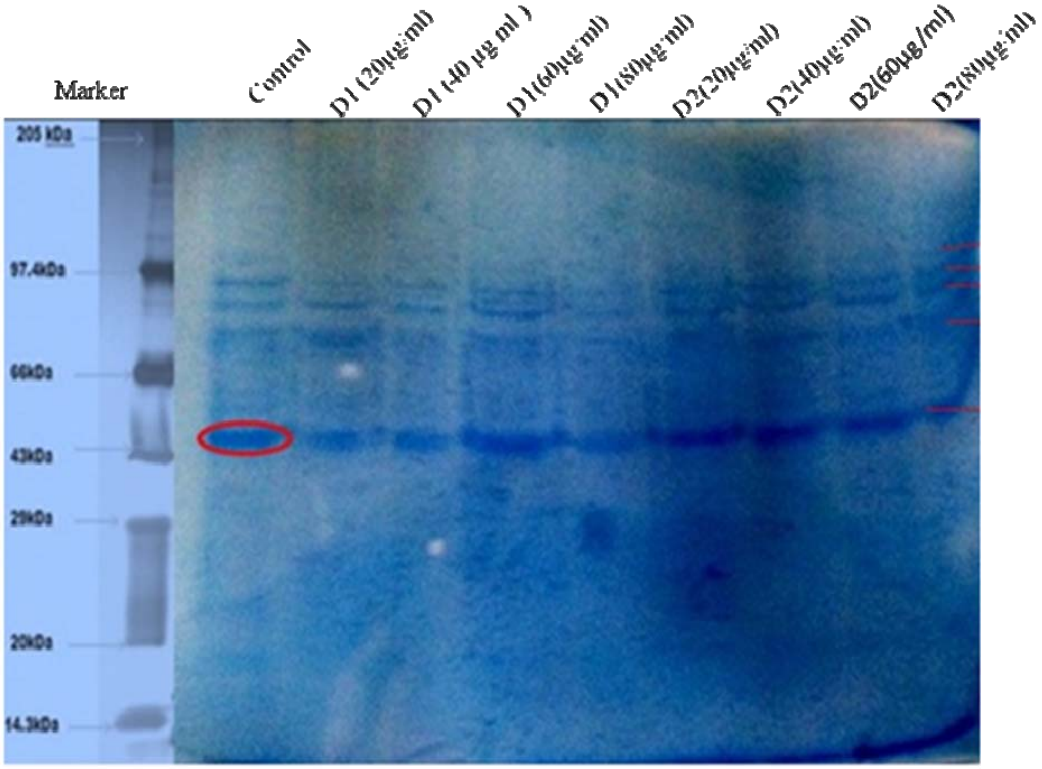


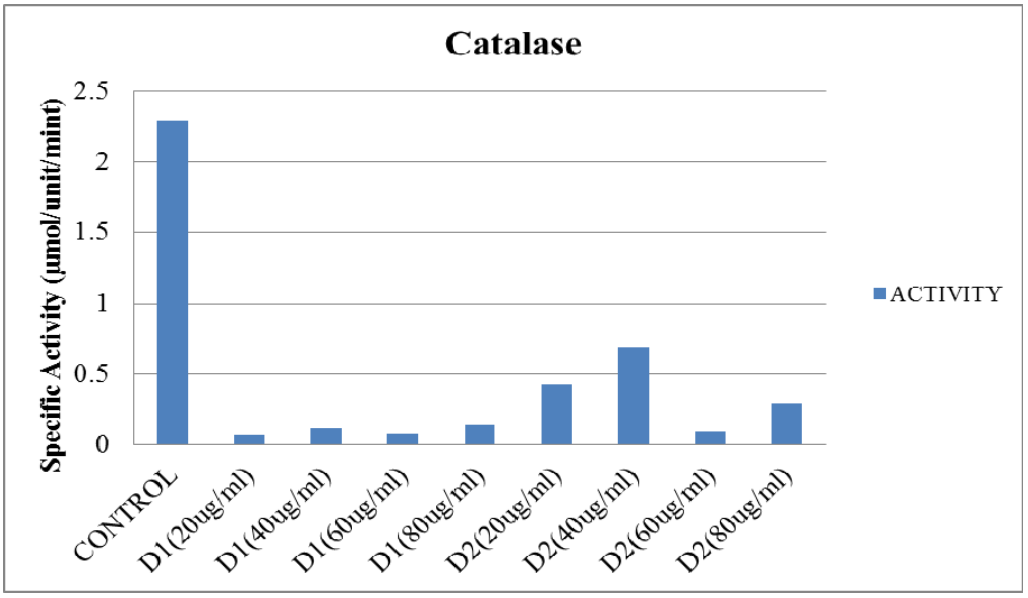
Figure 3: SDS-PAGE showing positive (lane 2) control with UV exposed after 30 minutes and with treatment of different concentration of D1 (lane 3,4,5&6) and D2 (lane 7,8,9 & 10) Drugs.

Biochemical Assay of the treated and exposed bacteria:

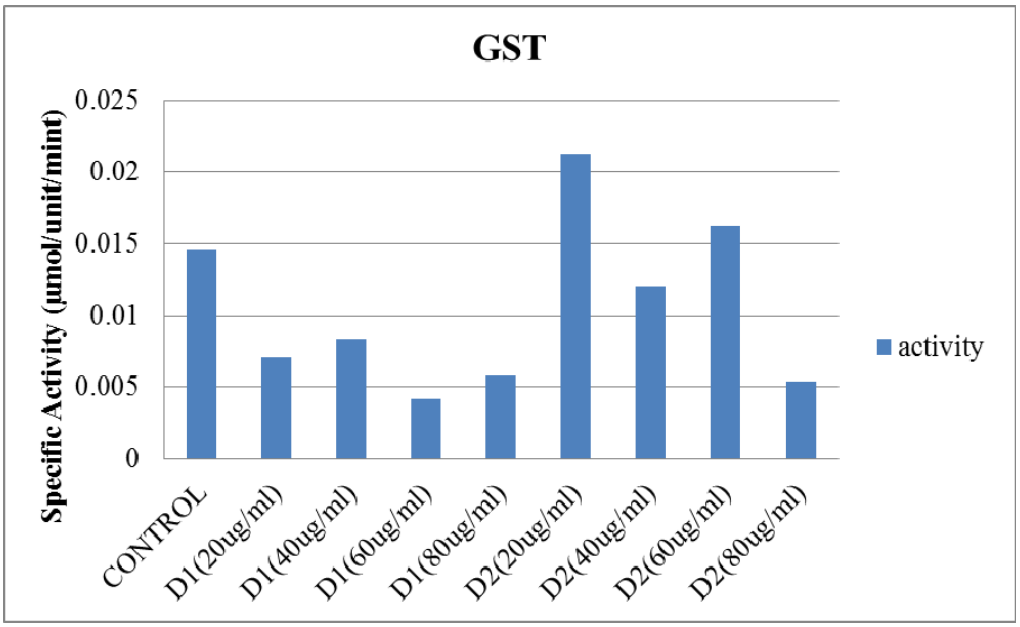
Catalase, GST and GSH were performed to analyse various enzymatic and non-enzymatic biochemical activity after 30 minutes of UV exposure for various concentrations of drug treatment, drug D1 and D2 as well as without treatment (control).

Catalase was found highest (2.3µmol/mint/ml) in the control sample which means that the control has more activity of catalase comparing to all the differentially treated (drug D1 and D2) and UV exposed DH5α. In drug treatment, D1 has more ROS at the conc of 80µg/ml which was showing 0.15µmol/min/ml activity and in D2, 40µg/ml has more ROS activity i.e. 0.7µmol/min/ml.

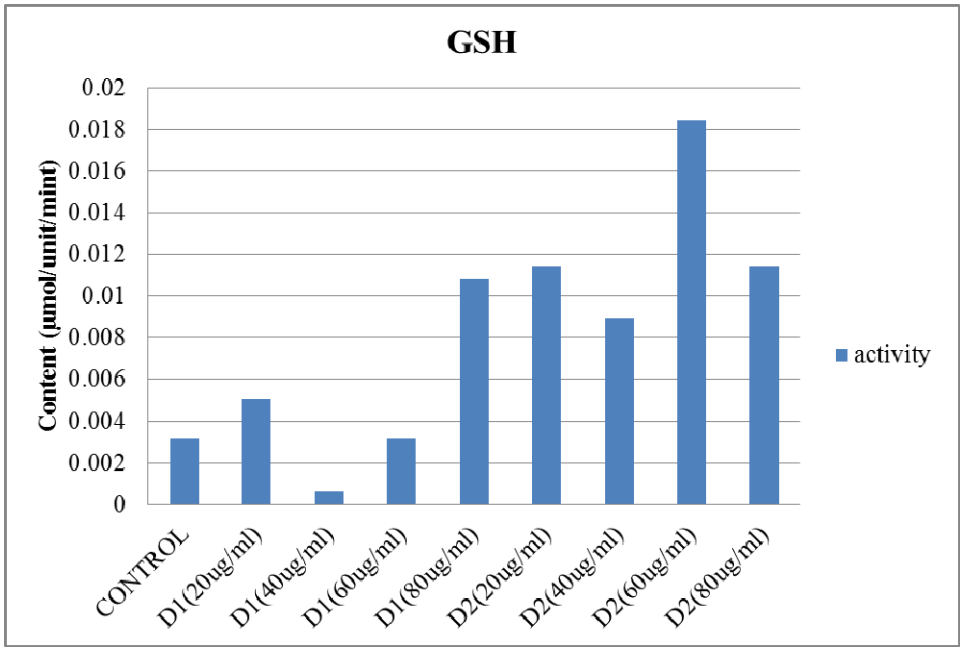
GST result has shown 0.0145µmol/min/ml activity in control and in drug D1 treatment, 40µg/ml concentration showed highest activity at 0.008 µmol/min/ml. and in drug D2, it was in the concentration 20µg/ml i.e. 0.021µmol/min/ml activity. However in GSH results, control has showed 0.003 µmol/min/ml activity. After drug D1 treatment, it was observed in the concentration of 80µg/ml, which was 0.011µmol/min/ml and with drug D2, it was observed at the concentration of 60µg/ml which showed 0.0185µmol/min/ml activity.



Graph 1: Catalase Activity after 30 minutes of UV exposure for various concentrations of drug treatment, drug D1 and D2.



Graph 2: GST Activity after 30 minutes of UV exposure for various concentrations of drug treatment, drug D1 and D2.



Graph 4: GSH content after 30 minutes of UV exposure for various concentrations of drug treatment, drug D1 and D2.

Formulation of the natural radio protectant cream

Colorless gel base was used for formulation which changes color from cream to yellow on addition of drug. Stability of the cream formulation was done by organoleptic examination.

Stability studies of the cream formulation

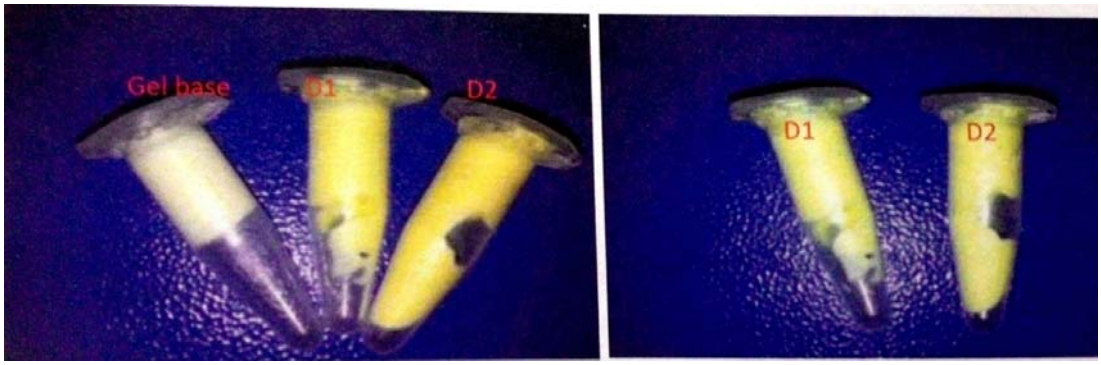


Figure 4: Showing Cream formulation of drug D1 and D2

Organoleptic study

Table 1: Observation table of organoleptic study at different temperatures

Characteristics	Room temp.	4 °C	40-45 °C
Colour	Yellow	Yellow	Yellow
Texture	Smooth	Smooth	Smooth
Smell	No change	No change	No change
Breaking	No	No	No
Contamination	No	No	No

No change in colour, texture, odour was observed. Also no contamination and breaking was observed in 7 days study.

pH measurements

The pH of natural gel base by which formulation was done and cream formulation was 6.31 and 6.72 respectively. After 5-7 days studies it's pH varies by ± 0.5.

Stability after centrifugation

Cream formulation was stable till 12,000 rpm but when centrifuged at 15,000 rpm, oil got separated from cream.

Viscosity

Prepared cream was not separated till 45-50 °C but when it further kept in temperature near about 60°C there is slight separation observed in the cream.

Spreadability

The parallel plate method was used to check cream formulation spreadabilty, the spreadabilty of 200 µl formulation was 5.5 cm aprox along with 100 gm weight.

Thermostatic stability

From -4 °C to 50°C the cream formulation was stable.

Discussion

It is a well-established fact that ionizing radiation at cellular level can induce damage in the biologically important macromolecules such as DNA, proteins, lipids and carbohydrates in the various organs. In some cells damage is expressed early, however in others damage is expressed over a period of time depending upon the cell kinetics and the radiation tolerance of the cells and tissues (Divya and Aiyavu, 2015).

Many plants are known to have beneficial therapeutic effects as noted in the traditional Indian system of medicine, Ayurveda. However, they have received little attention for their radioprotective nature as well as antioxidant activities. Medicinal plants can protect against harmful effects of ionizing radiations. Natural plant extracts or pure compounds have comparatively better therapeutic index as compared to chemically synthesized antioxidants (Tilak and Devasagayam, 2004).

It has been suggested by the Ayurveda that herbal formulations like mentat, abana, chyavanaprasha, rasayana, triphala, geriforteetc have protective effect against the harmful effects of ionizing radiations (Ashmawy et al., 2006; Scolastici et al., 2007).

It is well known that endogenous antioxidant enzymes like SOD and catalase act as endogenous defense mechanisms against the ROS-mediated biological damages. Any radioprotectant, thus, can exert its action by ROS scavenging and inducing the generation of the above enzymes in vivo (Divya and Aiyavu, 2015).

Damage is found both in eukaryotic as well as prokaryotic system, however first hand information can be obtained in prokaryotes as it is an easy model to handle. It has been found that prokaryotes have evolved various mechanisms to cope with UVR-induced damage. In the present study 20µg/ml of drug D1 has been found to have radioprotective activity as per spread plate method. DNA analysis studies of these plates has also shown no shearing as compared to positive control. However drug D2 at the concentration of 40µg/ml has shown the colony enhancement as well as DNA protectant activity by keeping the DNA intact on the gel.

It has been found by various researchers that E. coli also possesses a variety of glycosylases to repair oxidative DNA damage through the base excision repair pathway (Friedberg et al., 1995). In addition, several regulatory genes are involved in protecting cells from oxidative stress. For example, OxyR, a LysR family protein, can activate the transcription of genes involved in peroxide metabolism and protection (katG, ahpC, ahpF, and dps) and in redox balance (gor, grxA, and trxC) and genes encoding regulators, such as furand oxyS **(**Storz and Zheng, 2000). The E. coli SoxRSregulon provides defense against oxidative damage caused by superoxide anions. More than 10 genes, including nfo (endonuclease IV) and sodA (Mn-superoxide dismutase), belong to the SoxRSregulon (Greenberg et al., 1990; Tsaneva and Weiss, 1990). Sigma factor 38 (rpos) is another important regulator in E. coli in response to oxidative stress (Ivanova et al., 1997). Some genes that are under the control of OxyR are also regulated by RpoS (Eisenstark, 1998). Similar oxidative stress regulators have been identified in many other bacteria as well as pathogenic bacteria (Christman et al., 1985; Eiamphungporn et al., 2003; Nachin et al., 2001; Ochsner et al., 2000; Rocha et al., 2000; Ueshima et al., 2003).

In the similar pattern we have also observed many protiens getting upregulated and downregulated in the range between 43-97kDa, which are also depicted by HSPs and melanocytes proteins mentioned due to UV damage. DNA shearing has also been observed in the colonies of DH5α exposed for 30 and 60 min. However, shearing of DNA was less at concentration, 20µg/ml & 40µg/ml in drug D1 and 40µg/ml & 60µg/ml in drug D2 treated and UV exposed DH5α. It was prominent that 40µg/ml concentration of drug D2 in the ratio of 2:1 has been found to be most effective against UV radiation. Catalase was found to be highest in drug D2 at concentration of 40µg/ml i.e. 0.7µmol/min/ml. However GST and GSH were also found highest in drug D2 which was in concentration 20µg/ml i.e. 0.021µmol/min/ml and 60µg/ml i.e. 0.0185µmol/min/ml respectively.

On the basis of above finding we have concluded that the cream formulation of D2 at 40ug/ml concentration in the ratio of 2:1 (Berberine+Selenium) has potent radioprotective activity. Thus this formulation can be used as an effective radioprotectant cream.

Conflict of Interest: Nil

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