RESEARCH PAPER

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Radiation Sterilisation of Henna and its Ointment

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The feasibility of sterilisation of Henna and its ointment by gamma radiation is investigated. The drug and its ointment were irradiated at graded doses of 5-25 kGy and monitored for changes, if any, in physicochemical characteristics and safety of ointment. Microbiological studies were carried out to determine the minimum decontamination dose and sterility dose. Henna and its ointment were found to be sterile at 20 kGy dose. Studies did not reveal any untoward changes in the irradiated products upto 25 kGy dose of gamma radiation.

LEAVES of henna (**Lawsonia inermis**) are known to possess antibacterial, anti-inflammatory and astringent properties, that are necessary for burn healing, mainly due to the presence of lawsone (2-hydroxy-1,4-naphthoquinone), β- sitosterol and tannins respectively[2]. Being of natural origin, henna is likely to carry a high bioburden. Dried leaves of henna are exported in large quantities from India and exporting countries are required to furnish phytosanitary certificates[1]. Hence, there is a need for its decontamination.

An effective burn ointment of henna in polyethylene glycol (PEG) base is reported[2]. Burn ointments are required to be sterile. Heat sterilisation is not possible because of thermolability of the vegetable drug. Hence, gamma irradiation, a cold and terminal method of sterilisation, could be used as a promising alternative. Sterilisation of ointments in the final packaged container by gamma radiation has been reported[3].

The present investigation reports the feasibility of sterilising henna and its ointment by gamma radiation.

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EXPERIMENTAL

Materials

Lawsonia inermis leaves were procured from local market and correct botanical identity was established by comparing morphological and phytochemical characteristics with that reported for authentic sample.

All other chemicals and reagents used were of Anala R grade.

Preparation of henna extract

About 100 g of the powdered drug (#40) was macerated with 300 ml of 5% sodium bicarbonate solution for 24 hours and percolated with 700 ml of alcohol (95%) till completely exhausted. The extract was then concentrated by distillation under vaccum at 40° to 100 ml.

Formulation of henna ointment

Henna ointment of the following composition was prepared

PEG 1500	5.0 g
PEG 6000	2.5 g

Stearyl alcohol	2.5 g
Henna extract	4.0 ml
PEG 400 q.s	50.0 g

The base was prepared by fusion method[4]. Henna extract was incorporated in the base just before congealing by trituration process under clean conditions.

Irradiation

The powdered henna leaves (#40) were packed either in neutral glass vials or polythene bags. Henna ointment was filled in collapsible aluminium tubes (5 g) to 3/4th capacity. The samples were then irradiated by ^{60}Co gamma radiation at 5, 10, 15, 20 and 25 kGy at ISOMED, BARC, Bombay.

The control (unirradiated) and irradiated (25 kGy) samples of henna and its ointment were monitored for changes in various physicochemical characteristics.

Extractive values of henna were determined by successive solvent extraction in a series of solvents[5]. The total tannin content of henna extract was determined by Lowenthal's permanganate oxidation method[6,7].

For High Performance Thin Layer Chromatography, standard solution was prepared by dissolving lawsone (1 mg/ml) in alcohol (95%). Test solution was made by shaking the acidified henna extract with chloroform as described later under Lawsone content. Standard solution (5µl) and test solution (10µl) were then applied on precoated silica gel 60 F_{254} plates with Nanomat II. The chromatographs were developed in the mobile phase, 0.5N HCl : ethanol : acetone : 5 : 5 : 1 and scanned at 272 nm with TLC Scanner II on CAMAG HPTLC (Switzerland).

Electron spin resonance spectra of powdered drug samples were recorded on Varian E-line ESR spectrometer.

The samples of Henna ointment were observed for changes in colour, odour and appearance. The pH of aqueous solution (10% w/v) of the ointment was determined using an Elico pH meter, model LI-10. The ointment parameters like congealing point, spreadability and tube extrudability were determined using a freezing point apparatus[8], a sliding plate apparatus[9] and a lever-type apparatus[10] respectively. The rheological behaviour was assessed by Brookefield Synchrolectric Viscometer (model RV T) with spindle RV-7 at different shear rates. The primary skin irritation test of the ointment was performed on albino rabbits by patch test technique[11].

The Minimum Inhibitory Concentration (MIC) of henna extract and the antibacterial activity of henna ointment were determined by the agar disc diffusion method[12] and the agar cup plate method[13] respectively, against **Staphylcoccus aureus** (NCIM 2127), **Pseudomonas aeruginosa** (NCIM 2200) and **Escherichia coli** (NCIM 2067).

Determination of Lawsone content

Henna extract (1 ml) and 10 ml of 10% w/v aqueous solution of ointment were separately acidified to pH 4.5-5 with HCl and extracted with 25 ml of chloroform. The absorbance of each of the suitably diluted solutions was then determined on Bausch and Lomb spectronic 2000 spectrophotometer at 272 nm and lawsone content was calculated from the Standard Curve (2-10 mcg/ml).

Control and irradiated (graded doses) samples of henna and its ointment were tested for the total aerobic count, presence of objectionable microorganisms and sterility as per USP[14].

Stability testing

Control and irradiated (25 kGy) samples of henna and its ointment were stored at 5°, ambient conditions (28 ± 4)°, 37°, 45° and 60° (wherever applicable) and tested over a period of four weeks at weekly intervals for the content of active constituents and

Table 1: Effect of Gamma Radiation (25 Kgy) on Physicochemical Characteristics of Henna

Characteristics	Average Values*	
	Control	Irradiated
Extractive values (% w/w)		
Solvents		
Pet. Ether (60-80°)	3.197	3.201
Benzene	1.581	1.498
Chloroform	2.407	2.463
Acetone	5.596	5.683
Ethanol (95%)	8.851	8.961
Chloroform water	4.237	4.386
Lawsone content (mg/g)	1.69	1.68
Tannin content (mg/g)	11.13	11.34

*Average of six sets of experiments

Table 2 : Effect of Gamma Radiation (25 kGy) on Physicochemical Characteristics of Henna Ointment

Characteristics	Average Values*	
	Control	Irradiated
pH	7.1	7.1
Congealing point	45°	46°
Spreadability (secs)	12	14
Tube extrudability (mg)	380	300
Yield value (dynes)	7.2×10^3	7.8×10^3
Area of hysteresis loop (cm^2)	25	25
Consistency index (dynes. cms)	1364.583	1435.489
Flow index	0.1960	0.09803
Lawsone content (mcg/g)	137	136
Antibacterial activity zones of inhibition (cm)		
S. aureus	1.2	1.0
P. aeruginosa	1.7	1.6
E. coli	1.4	1.3

* Average of six sets of experiments.

antibacterial activity. Henna with elevated moisture content (10- 15% w/w) was subjected to irradiation and post-irradiation stability over a period of four weeks was studied.

RESULTS AND DISCUSSION

As shown in **Table 1**, there was no significant change in the extractive values in different solvents, lawsone content and tannin content of henna after irradiation. The MIC of extract of henna against **S. aureus, E. coli** and **P. aeruginosa** was found to be 0.04 ml which remained same even after irradiation. The HPTLC patterns for control and irradiated samples were found to be identical showing four peaks. ESR studies revealed that no free radical was produced during irradiation.

Physicochemical testing of irradiated (25 kGy) henna ointment revealed no significant change in the colour, odour, appearance, pH, lawsone content, antibacterial activity and congealing point when compared with control (**Table 2**).

The rheograms of control ointment showed pseudoplastic flow with thixotropy and there was no change after irradiation. No marked variation was observed in flow index, area of hysteresis loop and yield value (Table 2). The consistency index of irradiated ointment was increased by 5.19% as a result of which there was a decrease in spreadability and tube extrudability after irradiation. Some combinations of PEGs are reported to become slightly stiffer after irradiation[15].

The control and irradiated (25kGy) henna ointment caused no signs of irritation on intact or abraded skin patches of rabbits indicating non-irritancy of ointment even after irradiation. Similar findings are reported for PEG bases[15].

Henna was found to carry an initial bioburden of 10^4 c.f.u/g. It showed the presence of **S. aureus, E. coli** and **Salmonella**. The drug was satisfactorily decontaminated at 15 kGy and was found to be sterile at 20 kGy dose. The henna ointment having an initial bioburden of $4x10^3$ c.f.u/g and presence of **S. aureus** was found to be sterile at 20 kGy dose.

Thus, this study within the limits of the experimental design indicates the feasibility of decontaminating henna and its ointment by gamma radiation upto 25 kGy dose without significant changes in their physicochemical characteristics.

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