ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: www.ijrps.com

Design and Evaluation of Antimicrobial and Wound Healing Activities of Dual Loaded Flavones Nano Formulations Scaffold with Chitosan

Senthil Kumar C*, Praveen Kumar K N, Brintha N, Prakash G, Sundararajan N, Mohan S

Department of Pharmacology, Karpagam College of Pharmacy, Othakkal Mandapam, Coimbatore - 641032, Tamil Nadu, India

Article History:

Received on: 15 Sep 2020
Revised on: 18 Oct 2020
Accepted on: 19 Oct 2020

Keywords:

Anti-microbial activity,
Chitosan,
Flavonoids,
Quercitin,
Rutin,
Silibinin

ABSTRACT

Flavonoids belong to the comprehensive category of polyphenols and namely flavon-3-ols (flavonols, anthocyanins, isoflavones, flavanones and flavones) are found in plant sources. Quercetin, rutin and silibinin exhibit numerous pharmacological spectrum but it has hurdles like poor aqueous solubility, rapid clearance and poor penetration potential attribute to reduced clinical usage. Hence the aim of this research is to design and evaluate of antimicrobial and wound healing activities of dual loaded flavones nano formulations scaffold with chitosan-based film by nanopreception techniques. The prepared film was subjected characterization studies such as physical appearance, thickness, weight variation, folding endurance and water absorption capacity in addition was to evaluate anti-microbial activity of dual loaded flavones nano formulation film by well diffusion method and skin irritation study performed as per OECD 406 guide lines. The prepared duo loaded flavonol scaffold chitosan film employed with in-vivo wound healing activity in excision wound model. Results revealed that chitosan film scaffold with quercetin and rutin nano formulations and film scaffold with quercetin and silibinin nano formulations showed significant zone of inhibition when compared with control group. Acute skin irritation study revealed that the film scaffold with quercetin and rutin nano formulations and film scaffold with quercetin and silibinin nano formulations shows no adverse event such erythema and skin irritation. However, on 14th day, control group showed 75.91% wound healing, whereas the quercetin and silibinin group showed 84.75% wound healing. On the other hand, Quercetin and Rutin Group showed appreciable wound healing activity of 90.70%. Hence Chitosan scaffold film with quercetin and rutin nano formulations has enhanced wound healing and re-epitheliasation activity.

*Corresponding Author

Name: Senthil Kumar C
Phone: 9894105642
Email: drcskpharma@gmail.com

ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v12i1.4114

Production and Hosted by

IJRPS | www.ijrps.com

INTRODUCTION

Flavonoids are natural products widely shredded in the plant kingdom. They are polyphenols which are divided into six classes, specifically flavonols, flavanones, isoflavones, anthocyanins and flavones are found in plant sources. Flavonoids possess beneficial studies (Chidambaram *et al.*, 2013; Moorthi and Kathiresan, 2013). Among all flavonoids quercetin is one of the most abundant flavonoid and is widely distributed in nature. Quercetin is abundant in most plants, fruits and vegetables, which can reach levels

in the human diet as high as 16-25mg/day (Lakhanpal and Rai, 2007). Quercetin is a potent oxygen free radical scavenger and a metal chelator.

Quercetin being good antioxidant but with limited clinical application because of its hydrophobic nature and limited bioavailability. Poor absorption and extremely low distribution to wound area after administration due to both rapid metabolism and difficulties in penetration through the skin. Rutin is a bioflavonoid. Rutin and silibinin are bioactive molecules act as a bio enhancer which was abundantly found in the plant, and milk thistle is a part of the Asteraceae family. It is one of the most ancient herbal medicines, respectively. The pharmacological spectrum of rutin and its aglycone may differ, and the presence of rutinoside moiety is responsible for some of the protective effects of rutin.

Both bio-enhancers have their limitation, such as poor absorption because of less Lipophilicity and have low permeability across intestinal epithelial cells; rapid systemic clearance undergoes extensive first-pass metabolism respectively (Kostek *et al.*, 2012). To address the above limitation plan to encompassed the quercetin with bioenhancer rutin and silibinin scaffold with chitosan polymer using nano-drug delivery system.

MATERIALS AND METHODS

Chemicals utilized in this study were procured from various industrial sources and used as received without any modifications. Quercetin (QU), Rutin (RU) and Silibinin (Sigma Laboratories) Analytical grade 1% acetic acid, Poly ethylene glycol, Agar medium and Chitosan (polymer) was obtained from different Chemicals sources.

Methodology

Preparation of chitosan-based film scaffold with dual loaded flavono nanoformulation

0.2 gram of chitosan dissolved in 20ml of 1% acetic acid with constant stirring and mixed with 0.5ml of PEG (plasticiser). The mixture was dissolved by using a magnetic stirrer for about 4hrs and filtered by using musclin cloth. The drug was incorporated into the mixture. Spread the mixture in glass Petri plate and dry the mixture for about 24hours to form a biodegradable film (Tanwar, 2005).

Characterization of chitosan-based film scaffold with dual loaded flavono nanoformulation

Physical appearance

The film was examined visually for appearance, color, texture and presence of any clogging/precipitation.

Thickness

In order to detect the thickness, the film was studied using micrometer screw gauze.

Weight Variation

3 randomly selected circular films from each preparation were weighed individually to calculate the mean value.

Folding endurance

The film was exposed to repeated folded at the same place till it breaks or folded up to 100 times manually.

Water absorption capacity

The one-inch film was weighed and placed in 15ml of distilled water. The film was weighed at the first hour, second hour, third hour and 24th hour.

% water absorption capacity =

$$\frac{(Final\ weight - initial\ weight)}{Initial\ weight} \times 100$$

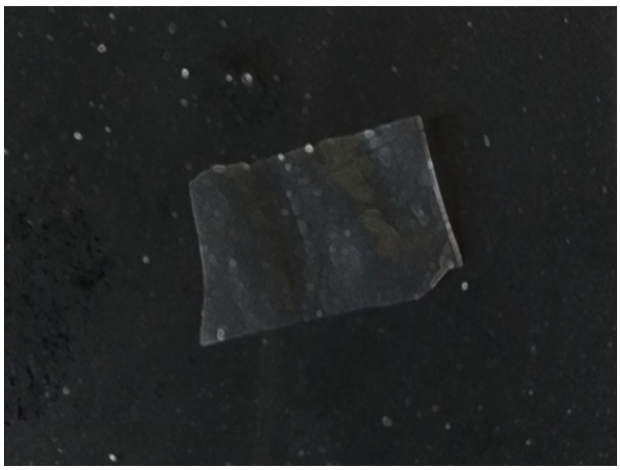

Figure 1: Chitosan-based film scaffold with dual loaded flavone nanoformulation

Evaluation of the anti-microbial activity of chitosan-based film scaffold with dual loaded Flavonol Nano Formulation

Nutrient agar medium was prepared and sterilized for 15mins in an autoclave at 121lbs. Agar plate was prepared in aseptic condition and plates were inoculated with *Escherichia coli* and *Staphylococcus aureus* strains using swab technique. Wells were created & drugs (control, 5μL, 10μL, 20μL) are diffused in the well. Then plates are incubated at 37°C for about 24hours, the zone of inhibition was observed and recorded (Hima *et al.*, 2010).

Acute dermal irritation test

The dorsal fur was clipped with electric clippers exposing a section of roughly 150 square centimeters (10x15cm2) approximately 24 hours before

Table 1: Characterization of Chitosan Based Film Scaffold with Dual Loaded Nano Formulation

Batch no	Thickness (mm)	Folding Endurance	Water Absorption Capacity (%)
1	0.15	120	110
2	0.11	105	105
3	0.11	100	105

Table 2: Wound healing activity

Groups	% wound contraction in different days in sq. mm		
	0 day	7th day	14th day
Control	22.20+4.44	59.80+2.95	75.91+2.10
T1 (Q+R)	32.50+4.84***	76.72+1.33*	90.70+0.69***
T2 (Q+S)	28.28+2.87**	68.29+1.49**	84.75+1.06***

treatment. The study was initiated by treating one rat. The animal was applied 1% film scaffold with dual loaded flavone Nano formulation which is that the test substance to the intact, clipped skin of one flank employing a metalline patch of 2x3cm. The patch was mounted on microspores tape, which was wrapped around the abdomen and secured with Coban patch.

In-vivo wound healing activity

Adult S. D albino rats weighing between 100-200 gm was obtained from the animal house of Karpagam Academy of higher education, Coimbatore. The animals were randomly split into 3 groups, each containing 6 animals and all studies were conducted in accordance with CPCSEA. Ethical committee clearance was obtained from IAEC.

RESULTS AND DISCUSSION

Preparation of chitosan-based film scaffold with dual loaded flavone nanoformulation

The image of prepared chitosan-based film scaffold with dual loaded flavone nanoformulation is shown in Figure 1.

Preliminary characterization of Trial Batches of Chitosan Based Film Scaffold with Dual Loaded Nano Formulation

The physical characterization results are interpreted below in Table 1.

In-vitro anti-microbial activity

The zone of inhibition was observed in the Film scaffold with Querctin and Rutin Nano formulations T_1 and Film scaffold with Querctin and Silibinin Nano formulations T_2 against two different bacterial species (*Escherichia coli* and *Staphylococcus aureus*) in agar disc diffusion technique.

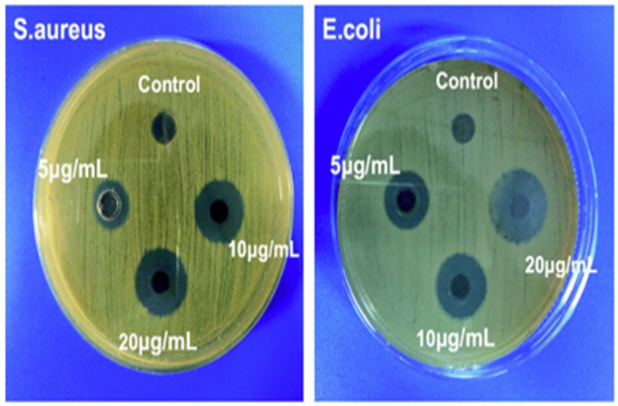

Figure 2: *In-vitro* anti-microbial activity

The two films showed significant antimicrobial activity than control.

Hence, film scaffold with Querctin and Rutin Nano formulations T_1 and Film scaffold with Querctin and Silibinin Nano formulations T_2 has antimicrobial activity (Figure 2).

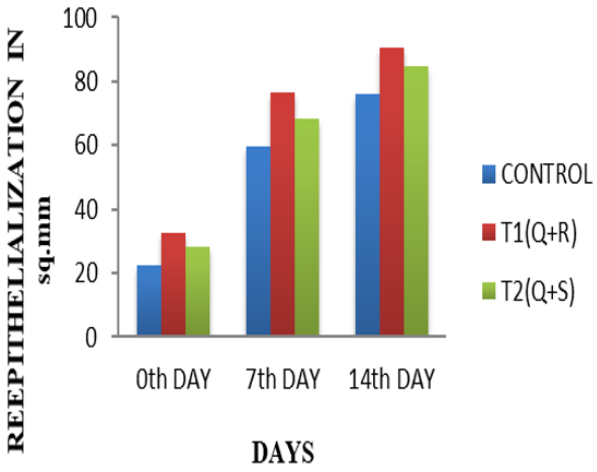

Figure 3: Percentage of wound contraction

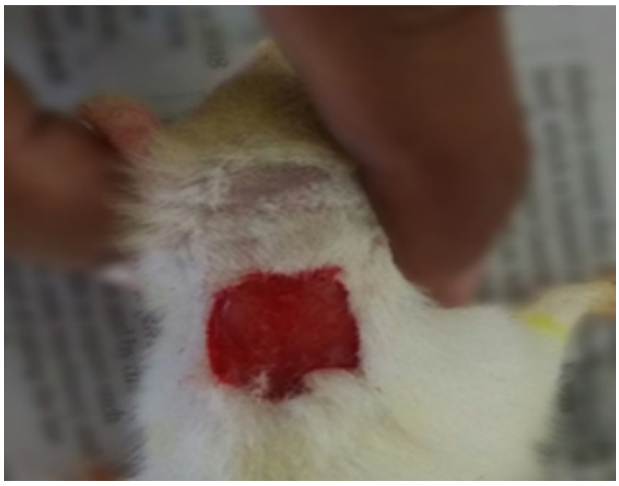

Figure 4: Control at Day 0

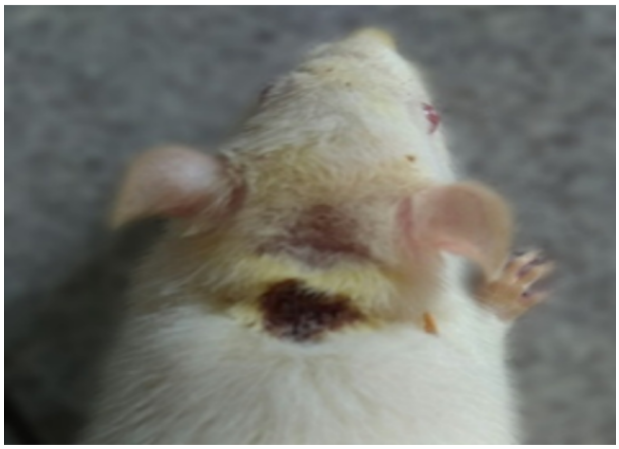

Figure 5: Control at Day 7

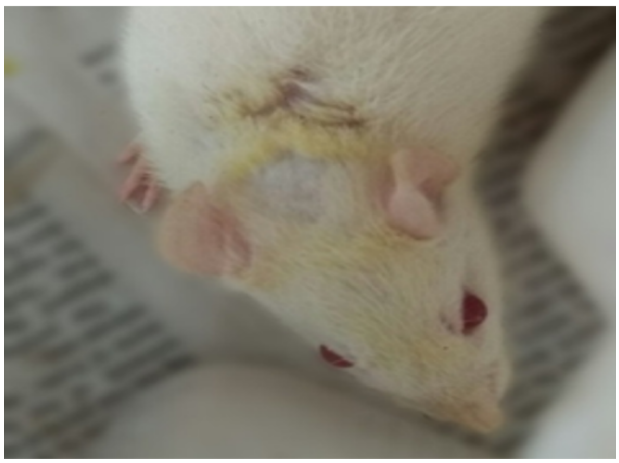

Figure 6: Control at Day 14

Figure 7: T1 (Q+R) at Day 0

Figure 8: T1 (Q+R) at Day 7

Figure 9: T1 (Q+R) at Day 14

Figure 10: T2 (Q+S) at Day 0

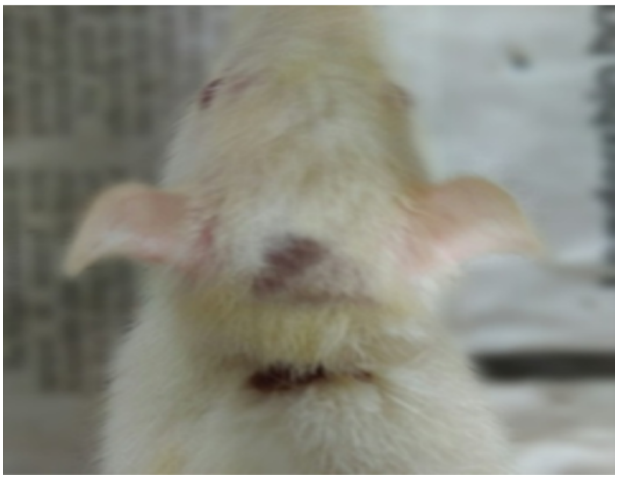

Figure 11: T2 (Q+S) at Day 7

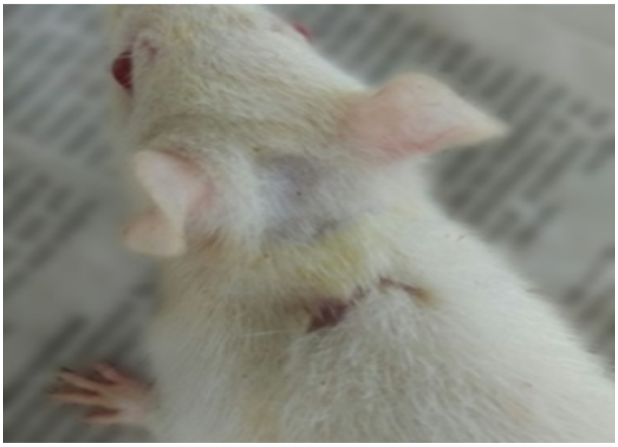

Figure 12: T2 (Q+S) at Day 14

In-vivo wound healing activity

Film scaffold with Querctin and Rutin Nano formulations T_1 showed impressive wound healing activity compared to wound healing observed in control group rats. Film scaffold with Querctin and Silibinin Nano formulations T_2 showed significant wound healing activity. Hence, film scaffold with Querctin and Rutin Nano formulation T_1 showed much more significant wound healing and reepitheliasation activity compare to that of Film scaffold with Querctin and Silibinin Nano formulations T_2 (Table 2, Figure 3, Figure 4, Figure 5, Figure 6, Figure 7, Figure 8, Figure 9, Figure 10, Figure 11, Figure 12).

It was further found that all three groups showed a reduction in wound area from day to day. However, on 14^{th} day, the group-I showed 75.91% protection, (which may be due to self-immunity of animals) whereas the group-III showed 84.75% protection. On the other hand, group-II showed appreciable wound healing activity of 90.70**%** protection. Hence film scaffold with Querctin and Rutin Nano formulations T_1 has enhanced wound healing and reepitheliasation activity.

CONCLUSIONS

From the study, it is concluded that the Chitosan scaffold film with quercetin and rutin nanoformulations demonstrated enhanced wound healing and reepitheliasation activity.

Funding Support

The authors declare that they have not received any kind of funding support.

Conflict of Interest

The authors declare they do not possess any conflict of interest.

REFERENCES

Chidambaram, M., Kumar, C. S., Mohan, S., Krishnan, K., Kathiresan, K. 2013. Application of validated RP–HPLC–PDA method for the simultaneous estimation of curcumin and piperine in Eudragit E 100 nanoparticles. *Journal of Pharmacy Research*, 7(3):224–229.

Hima, B. T., Vidyavathi, M., Kavitha, K., Sastry, T. P., Suresh, K. R. 2010. Preparation and evaluation of ciprofloxacin loaded chitosan-gelatin composite films for wound healing activity. *International Journal of Drug Delivery*, 2(2):173–182.

Kostek, H., Szponar, J., Tchórz, M., Majewska, M., Lewandowska-Stanek, H. 2012. Silibinin and its

hepatoprotective action from the perspective of a toxicologist. *Przeglad lekarski*, 69(8):541–543.

Lakhanpal, P., Rai, D. K. 2007. Quercetin: A Versatile Flavonoid. *Internet Journal of Medical Update - EJOURNAL*, 2(2):22–37.

Moorthi, C., Kathiresan, K. 2013. Curcumin–Piperine/Curcumin–Quercetin/Curcumin–Silibinin dual drug-loaded nanoparticulate combination therapy: A novel approach to target and treat multidrug-resistant cancers. *Journal of Medical Hypotheses and Ideas*, 7(1):15–20.

Tanwar, Y. S. 2005. Formulation and evaluation of transdermal films of salbutamol sulphate. *Dhaka Univ J Pharm Sci*, 4:93–100.