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Original Research Article

Efficacy of Hydrogel Containing Rutin in Wound Healing

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Abstract: With recent research in novel drug delivery formulations to provide stable and economical drug delivery systems, the pivot is on hydrogel which are known to minimize the problems of not only conventional dosage forms but also of novel drug delivery systems. Hydrogel is a biocompatible, convenient and stable drug delivery system for molecules as small as NSAIDs (Non-steroidal anti-inflammatory drugs) or as large as proteins and peptides. Healing of wounds begins from the moment of injury and can be extended for varying periods of time depending on the degree of wounding and the process of wound healing can be broadly classified into three stages; inflammatory phase, proliferate phase, and finally the remodeling phase which finally governed the strength and appearance of the healed tissues. Research had been recognized flavonoids to be an exceptional class of therapeutic molecules due to their versatile therapeutic potential. Rutin, class of flavonoid (vitamin P) has been explored for a numeral of pharmacological effects. In the present work attempted was made to determine the efficacy of rutin in hydrogel drug delivery system for the evaluation of wound healing activity. The drug content of the formulation H2 (0.025%w/w) was less than H3 (0.030%w/w), therefore H2 formulation was considered as best formulation.

Keywords: Hydrogel, Rutin, Flavonoids & Wound healing.

INTRODUCTION

Herbal drugs play an essential role in health care programs especially in developing countries. Ancient Indian literature incorporates an extremely broad definition of medicinal plants to be potential sources of medicinal substances [1]. Hydrogel is a hydrophilic mixture having the properties of both solid and liquid. The hydrogel can be defined as a cross linked polymeric matrix which has the capacity to hold water inside its porous design. The water holding capacity of the hydrogels arise mainly due to the presence of hydrophilic groups, *viz.* carboxyl, amino and hydroxyl groups, in the polymer chains. Hydrogels are cross-linked polymeric networks and consequently provide the hydrogel with a 3-dimensional polymeric network structure. Apart from the synthetic polymers, viz. PHEMA and poly (methyl methacrylate) (pMMA), the use of natural polymers, also termed as biopolymers, for the development of hydrogels have acquire a valuable importance over the years. Chitosan and Alginate are the two biopolymers which have been comprehensively studied in the recent past. The use of hydrogel is not only narrow to pharmaceutical and nutraceutical delivery but has also been expanding to regenerative medicine. Hydrogels has ability to absorbing large quantities of water without dissolving. Softness, smartness, and water storing capacity build hydrogel unique materials [2].The ability of hydrogel to absorb water arises from hydrophilic functional groups attached to the polymer backbone while their confrontation to dissolution arises from cross-links between network chains. Water inside the hydrogel enables free diffusion of solute molecules, whereas polymer serves as a matrix to grasp water together [3]. Hydrogels act as a moist wound dressing medium and have the ability to absorb and retain the wound exudates along with the foreign bodies, such as bacteria, within its network structure. In addition to this, hydrogels have been found to encourage fibroblast proliferation by minimized the fluid loss from the wound surface and protect the wound from external harm necessary for rapid wound healing [4]. Wound infection is one of the most recurrent diseases in developing countries because of poor hygienic conditions. Wound is a break in the epithelial coherence of the skin and may be leading by disruption of the structure and function of underlying normal tissues and may also result from a haematoma, contusion, laceration or an abrasion. Healing of wounds

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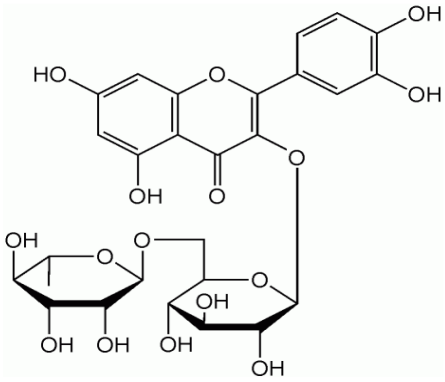
begins from the moment of injury and can be extended for varying periods of time depending on the degree of wounding and the process of wound healing can be broadly classified into three stages; inflammatory phase, proliferate phase, and finally the remodeling phase which finally governed the strength and appearance of the healed tissues [5].

Phases of Wound Healing [6]

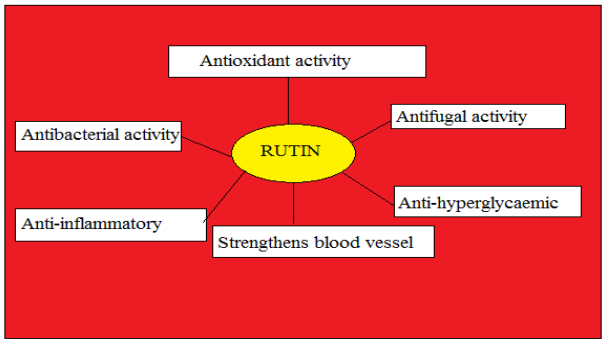
Flavonoids are universal in photosynthesizing cells and are often found in vegetables, fruit, nuts, seeds, stems, tea, flowers, honey and wine preparations. Due to versatile nature of flavonoids; they act as the principal physiologically active constituents have been used to treat human diseases since past decades. This class of natural products is becoming the subject of anti-infective research, and many groups have isolated and identified the structures of flavonoids possessing, antiviral, antifungal and antibacterial activity. Rutin by acting as antioxidants exhibited several beneficial effects, such as anti-inflammatory, anti-allergic, antiviral as well as an anticancer activity. They have also been suggested to play a protective role in liver diseases, cataracts, and cardiovascular diseases.



Structure of Rutin

Pharmacological action of Rutin

Rutin showed the most potent inherent activity, and produced the strongest inotropic responses among the different flavonoids. It exhibits antiulcer activity. Rutin is effective against cirrhosis i.e. hepto-protective. Other pharmacological potential was summarized [7].



In the present work attempted was made to determine the efficacy of rutin in hydrogel drug delivery system for the evaluation of wound healing activity.

MATERIAL AND METHODS

Material

Rutin was previous isolated from leaves of *A.squamosa* and characterized by chromatographic as well as various modern analytical methods like FT-IR, NMR & Mass spectroscopy.

Selection of polymer

The polymers were selected on the basis of the drug excepient compatibility studies. The drug excepient compatibility of the Rutin with Carbopol 934 & HPMC was determined by FT-IR spectroscopic studies (Brucker).

Experimental Protocol

Preparation of Hydrogels

The hydrogel forming polymers were dissolved in small amount of double distilled water in various proportions as shown in Table no.1 and then glycerine and sodium benzoate were added. The methanolic dispersion of rutin (1mg/ml) was added to it and the volume was made up to 100 ml. Then, sonicated (Lark probe sonicator) at 6φ frequency, 20 sec at 28^0C. The above formulation was allowed to stand for 24 hr at room temperature. The pH of this gel preparation was maintained 6 ±0.4 and stored in well closed container.

Characterization of Hydrogels

Various parameters like appearance, pH, viscosity, spread ability & % entrapment of formulated hydrogel (H1, H2 & H3) were studied.

Drug content determination

Rutin content in hydrogel was assayed by RP-HPLC method.

Selection and procurement of animals

Albino rats were procured and rats of either sex (weighing 150-200 g) were selected, maintained at 24-28^0C, housed individually with free access to food and water. Rats were fed standard diet and kept in well-ventilated animal house with alternate dark-light cycle of 12 hrs throughout the studies (CPCSEA-1323/10-CPCSEA).

***In – vivo* Evaluation for Wound Healing Activity**

Selection and procurement of animals

Albino rats were procured and rats of either sex (weighing 150-200 g) were selected, maintained at 24-28^0C, housed individually with free access to food and water. Rats were fed standard diet and kept in well-ventilated animal house with alternate dark-light cycle of 12 hrs throughout the studies (CPCSEA-1323/10-CPCSEA).

To perform the experiment, the mice were divided into seven groups consisting of six animals each.

Group I - CONTROL
Group II - TEST (H1)
Group III - TEST (H2)
Group IV - TEST (H3)
Group V - Standard [Standard Povidone-Iodine (5% w/w) Ointment]

EXCISION WOUND MODEL

For the excision wound study, each group containing six animals were selected circular wound of about 2.5 cm diameter were made depilated dorsal thoracic region of rats were deplicated under light ether anaesthesia in semi-aseptic condition and observed throughout the study. Individually rats were kept in housed. The different groups were divided and treated with hydrogels systems on the wound once daily for 14 days starting from the day of wounding. The observations of percentage wound closures shall be made on 4^{th}, 8^{th}, 12^{th} and 16^{th} post wounding days and also epithelization, size and shape of scar noted. All the samples were applied once daily for 16 days, starting from the day of wounding and evaluated for the following parameter [8].

The percentage wound contraction was determined using the following formula

$$\% \text{ Closure} = \frac{\text{Wound area on corresponding days - Wound area on day zero X 100}}{\text{Wound area on day zero}}$$

INCISION WOUND MODEL

Under light ether anaesthesia, two paravertebral incisions of 6 cm were made on either side of the vertebral column through the entire thickness of the skin with the help of sharp blade. The incisions were sutured using Ethicon 4-0 silk thread with the help of straight round bodied needle. Sutures shall be removed on 8^{th} post wounding day and the tensile strength shall be determined on 10^{th} post wounding day by using tensiometer.

Measurement of tensile strength

Tensile strength is the resistance to breaking under tension. For the newly repaired tissue including scar was excised to measure the tensile strength using Tensiometer [8].

Table-1: Composition of Hydrogel formulations

S.No.	Components	Quantity		
		H1(2:1)	H2(1:1)	H3(1:2)
1.	Carbopol 934	500 mg	500mg	250 mg
2.	HPMC	250 mg	500mg	500 mg
3.	Acaica	500mg	500mg	500mg
4.	Glycerine	2ml	2ml	2ml
5.	Sodium benzoate	100mg	100mg	100mg
6.	Rutin	4mg	4mg	4mg
7.	Distilled water	25 ml	25 ml	25 ml

Table-2: Characterization of formulated Hydrogels

S.No.	Characteristics	Results		
		H1	**H2**	**H3**
1.	Appearance	glossy, pale-yellowish coloured	glossy, pale-yellowish coloured	glossy, pale-yellowish coloured
2.	pH	5.5	5.7	5.7
3.	Viscosity	4200cps	4000cps	3600cps
4.	Spreadability	5.1 g.cm/sec	5.6 g.cm/sec	5.02 g.cm/sec
5.	% entrapment	97.2%	99.01 %	97.51%

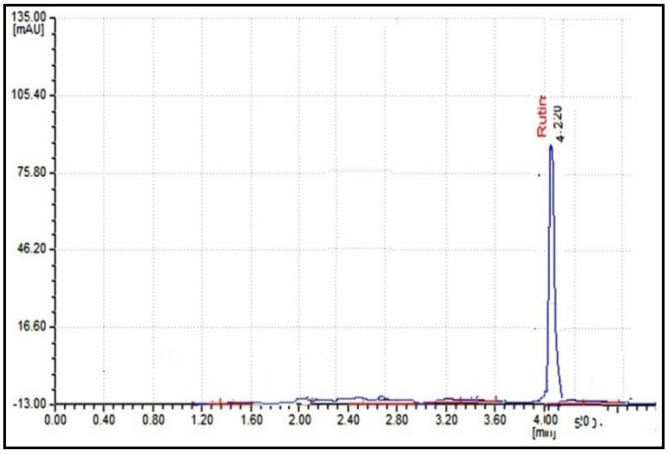


Fig-1: HPLC chromatogram of standard rutin

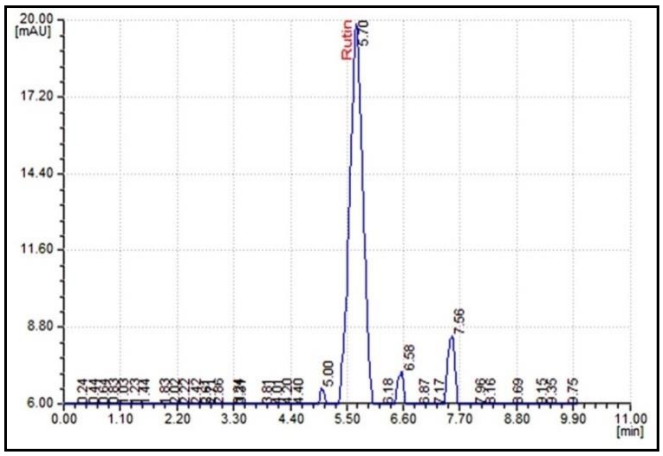


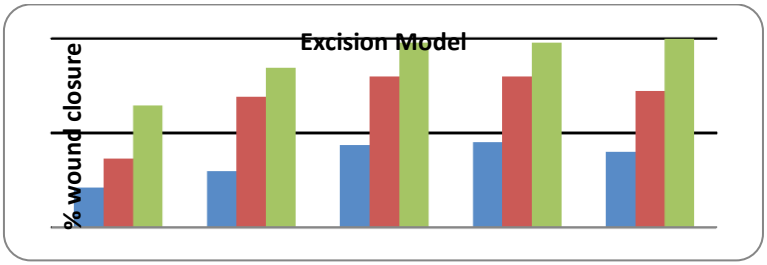
Fig-2: HPLC chromatogram of Hydrogel containing rutin

Excision Wound Model

Table-3: Mean Percentage closure of Excision wound area by different formulations

Groups	Mean Percentage closure of Excision wound area					Period of epithelization
	Initial	4thday	8thday	12thday	16thday	
Control	500	395.45±1.52 (20.91%)	318.14±0.63 (36.42%)	176.10±0.69 (64.78%)	124.30±0.60 (75.14%)	23 day
H1(0.020%w/w)	452.57±0.81	318.02±0.84 (29.73%)	140.251±0.75 (69.01%)	70.73±0.27 (84.37%)	18.05±0.03 (96.01%)	18 day
H2(0.025%w/w)	314.28±0.90	176.78±0.79 (43.75%)	63.64±0.29 (79.75%)	7.07±0.11 (97.75%)	—	15 day*
H3(0.030%w/w)	491.07±0.56	269.59±0.37 (45.1%)	98.16±0.12 (80.01%)	9.82±0.01 (98%)	—	15 day
Standard	210.2	126.12±0.23 (40%)	58.015±0.11 (72.4%)	0.378±.024 (99.82)	-	13 days*

Values are expressed as Mean ± SEM, n = 6 in each group, P< 0.001 significance Vs control



Graph-1: Mean % wound closure by different hydrogel formulations

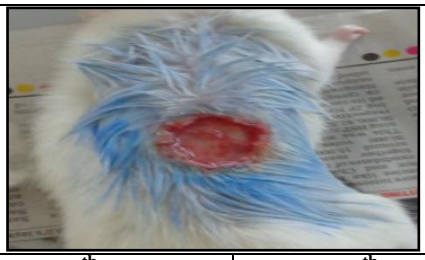
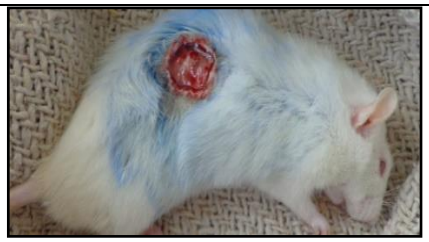
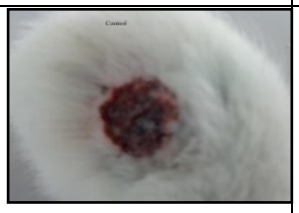
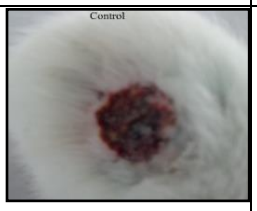
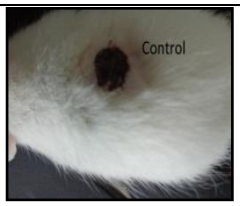
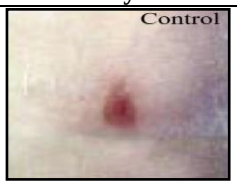
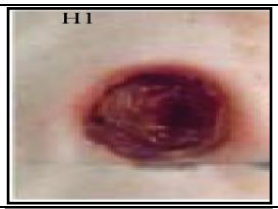
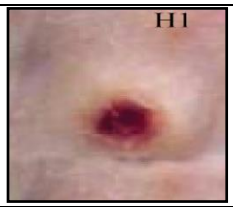
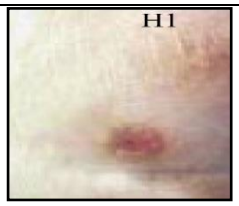
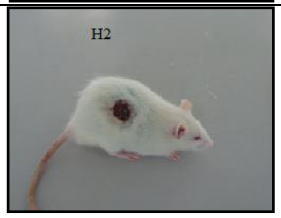
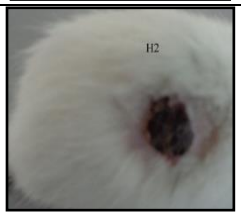
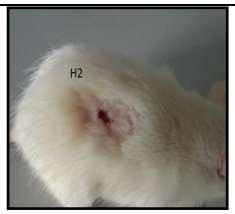
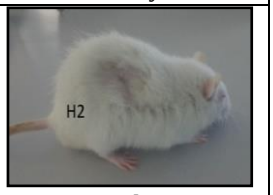
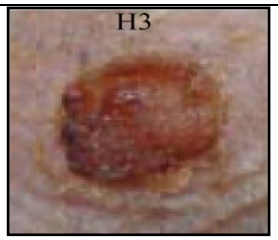
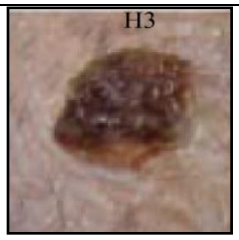
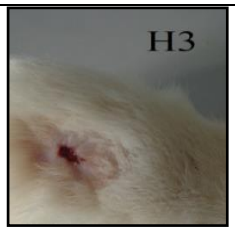
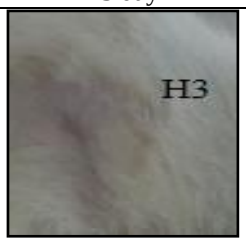
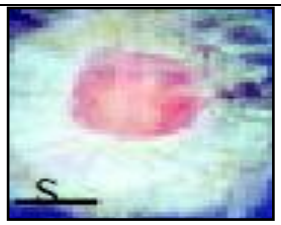
Initial wound creation				
Days	**4th**	**8th**	**12th**	**Day***
Control				 20 day
Formulation H1				 20day
Formulation H2				 15 day
Formulation H3				 15 day
Standard				 11 day

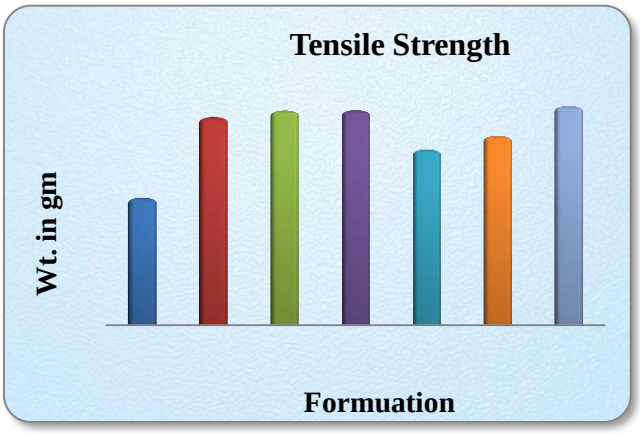
Fig-3: Excision wound Model Fig-3: Excision wound Model

Fig-4: Incision model

Table-4: Mean Tensile strength of resutured Incision wound on 10th Post Wounding Day

S. No.	Groups	Breaking strength (gm)
1.	Control	244.86 ± 1.47
2.	H1	398.2±0.36
3.	H2	410.42±0.79*
4.	H3	410.96±01.29
5.	Standard	419.2±3.8

Value are expressed as the Mean ± SEM, n = 6 in each group P< 0.001 significance Vs control



Graph-2:Tensile strength of different hydrogel formulations

RESULT AND DISCUSSION

In this research work Hydrogels containing Carbopol 940 as a polymer with different concentration of rutin was prepared (table 1 & 2) and efficacy for wound healing was determined by different models. For evaluation of wound healing potential of different hydrogel formulations various parameters like wound contraction and tensile strength Table 3 and fig.3 represents the reduction of wound area of the different groups over the period of 4th, 8th, 12th, 16th days. It had been seen that fastest wound healing took place in case of animals treated with H2 and H3 formulations. The % wound contractions were found to be 97.75% and 98%, respectively at 12th day. For both the formulations the period of epithelization was 15 days. The drug content of the H2 (0.025%w/w) was a lesser amount of than H3 (0.030%w/w), so consequently H2 formulation was considered as best formulation. The least rate of wound healing was seen in control group, which received no treatment. As compared with standard the tensile strength was also found to be best in H2 formulation (table 4 and graph 2). Flavonoids prove wound healing properties due to their antibacterial and antioxidant properties. Flavones, flavonoids and flavonol are phenolic structure with single carbonyl group. Flavonoids are recognized to promote the wound-healing process mainly due to their antimicrobial potential, which appear to be accountable for wound contraction and increased rate of epithelialization. Flavonoids are recognized to reduce lipid peroxidation not only by preventing or slowing the onset of cell necrosis but also by improving vascularity. In addition to this, hydrogels have been found to promote fibroblast production by reducing the fluid loss from the wound surface and protect the wound from external noxae necessary for rapid wound healing. Carbopol based hydrogels were selected as delivery system for wound treatment. Among the formulations to be useful on damaged skin, hydrogels have shown the superiority as

they can provide a moist environment for the wound and at the same time deliver the incorporated drug to the wound [10].

CONCLUSION

Topical hydrogel formulations containing rutin was screened for treatment of skin wound. Wound healing potential of different hydrogel formulations was assessed through various parameters like wound contraction &tensile strength were assayed. On the basis of the results obtained in the present investigation, it was found that formulation H2 (0.025%w/w rutin) was considered to best formulation. The above findings justify the wound healing potential of hydrogel containing rutin as well as the isolated moiety rutin. A lot of research has been carried out on wound healing .Synthetic drugs have side effect like nausea, vomiting, epigastric distress, peptic ulceration, diarrhea, edema, Stevens–Johnson syndrome etc. So, it has been found that herbal formulations have beneficial effect on human being, in order to minimize these side effects over synthetic drugs. Flavonoids are known to endorse the wound-healing progression mainly due to their antimicrobial potential, which appear to be liable for wound contraction and increased rate of epithelialization. Flavonoids are predictable to reduce lipid peroxidation not only by preventing or slowing the onset of cell necrosis but also by improving vascularity [9]. Hydrogels are applied to the wound as gels; they required a second cover such as gauze. Besides, if they are applied as films to the wound area, they can be used both as a primary and secondary dressing [11, 12]. So hypothetical mechanism of healing is that flavonoid (Rutin) along with suitable drug delivery system causes synergetic effect on wound and promote rapid tissue repair.

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