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Physicochemical properties of rutin loaded into nanoliposomes and its uses for the treatment of oral ulcers

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Oral ulcers cause pains and inflammation, and an appropriate ointment must be used for their treatment. Rutin is a flavonol with antibacterial and anti-inflammatory properties that can be used to treat ulcers. Encapsulation of rutin increases its stability and prevents its degradation. This study was conducted to investigate the physicochemical properties of rutin loaded into nanoliposomes and its uses for the treatment of oral ulcers. Nanoliposomes were prepared with the help of Phospholipid 1,2-dioleoylsn-glycero-3-phosphoethanolamine and cholesterol, and their physicochemical properties of particle size, zeta potential, encapsulation efficiency, and in vitro release were investigated. The ulcers were induced on rats and treated with ointments lacking rutin and containing 5, 10, and 15 µg/mL rutin. The healing rate, edema, and the serum concentrations of interleukin-2 (IL-2), tumor necrosis factor-α (TNF-α), and vascular endothelial growth factor (VEGF) were investigated at the end of the treatment period. The nanoliposomes prepared from rutin did not show significant differences for particle size and zeta potential ($p>0.05$) while loading rutin increased encapsulation efficiency and prolonged in vitro release. The ointments prepared from rutin not only decreased edema but increased the serum concentration of VEGF and decreased the serum concentrations of IL-2 and TNF-α ($p<0.05$). In conclusion, ointments prepared from rutin showed an excellent potential for treating oral ulcers in animal models that must be considered.

KEYWORDS

Particle size; inflammation; animal model; oral ulcers; edema.

Introduction

Oral ulcers are lesions created by losing mucosal tissue in the oral cavity [1]. The ulcers are oral mucosal disorders with a high prevalence that are appeared in periodic intervals [2]. Various factors induce oral ulcers, such as trauma, microorganisms, allergies, drugs, and systematic disorders [3]. Oral ulcers cause pains in the nerve ends of lamina propria [4]. Oral ulcers cause problems for chewing, swallowing, and speaking that influence patients' quality of life [5]. Ulcer tissue is prone to infection and causes inflammation and tissue necrosis [2]. Several inflammatory factors are involved in the healing process of oral ulcers. Interleukin-2 (IL-2) is involved in immunity and inflammatory responses [6]. Tumor necrosis factor-α (TNF-α) increases vascular endothelial cells' permeability and activates lymphocyte and IL-2 [2]. Vascular endothelial growth factor (VEGF) increases protein

infiltration and modulates the wound healing process [7]. Agents used to treat wounds must adhere on wound region and have anti-inflammatory and antibacterial properties [8]. Herbal medicines and their derivations are broadly used to treat ulcers due to their biological properties [9-12].

Rutin (3,30,40,5,7-pentahydroxyflavone-3-rhamnoglucoside) is a flavonol found in plants. It is composed of flavonol aglycone quercetin and rhamnosyl glucose [13]. Rutin is known to have antioxidant properties and scavenging free radicals [14,15]. Other properties of rutin include anti-inflammatory, antimicrobial, anticarcinogenic, and neuroprotective [16]. Despite all the advantages, rutin has disadvantages such as less solubility in an aqueous phase and polarity that decrease the biological properties of rutin [17]. It is also prone to degradation that decreases its efficiency. To overcome on limitations, studies have suggested methods such as encapsulation.

Nanotechnology is used in various fields such as nanosensors, nano delivery systems, and nanocomposite packaging. Nanoliposomes have a greater surface area and stability profile that maintain their size within nanometric scales [18]. They are composed of lipids and phospholipids. Nanoliposomes have an amphiphilic nature that helps them entrapping and release a massive range of hydrophilic and hydrophobic compounds [19].

Thus, rutin loaded into nanoliposomes may improve the healing process in oral ulcers in rat models. This preliminary study investigates the physicochemical properties of rutin nanoliposomes and their uses for treating oral ulcers.

Materials and methods

Materials

Rutin was purchased from Sigma–Aldrich Company (Saint-Quentin Fallavier, France). Phospholipid 1,2-dioleoylsn-glycero-3-phosphoethanolamine and cholesterol were prepared from Avanti Polar Lipids (Alabaster, AL, USA).

In vitro studies

Preparation of rutin nanoliposomes

Nanoliposomes were prepared with the help of Phospholipid 1,2-dioleoylsn-glycero-3-phosphoethanolamine and cholesterol and in concentrations of 0, 5, 10, and 15 µg/mL, as reported by previous studies[20].

Physiochemical properties

The particle size and zeta potential were investigated by a Zetasizer nano ZSP (Malvern Instrument, Worcestershire, UK). Encapsulation efficiency and in vitro release were investigated as reported by previous studies [21].

In vivo studies

Animals and keeping condition

Sixty male adult rats were used in this study; the rats had a weighted mean of 183.52 ± 20.56 g and 12 weeks of age. Male rats had unlimited access to water and feed and a lighting diet of 12 h darkness and 12 h lightness. Animals were adapted to the environment for 14 days, and then experiments were started. Animals were kept at 20–25 °C and relative humidity 40–60%.

Induction of ulcer and treatment

Ulcer was induced as reported by previous studies after induction of anesthesia [2]. The rats were prepared with Triamcinolone ointment (Iran Darou Company, Tehran-Iran) without rutin (control) and containing 5 (5-R), 10 (10-R), and 15 (15-R), µg/mL rutin for seven days. Rutin (2 g) was added to 98 g Triamcinolone ointment. Ointments were administrated once/day for five days.

The investigation of ulcer parameters

At the end of the study, the rats were investigated for the healing rate, and the number of healed rats was calculated. The rats with ulcers lesser than 1 were considered healed while those with ulcers higher than 1 were considered non-healed. At the end of the week, blood samples were collected from five rats per group, and sera were analyzed for serum levels of TNF-α (ab46070), IL-2 (ab221834), and VEGF (ab100786) as suggested by the producer Company (AbCam Company, USA). Edema was classified as hyperemia around ulcer: I degrees: hyperemia diameter <1 mm; II degrees: hyperemia diameter 1–2 mm; III degrees: hyperemia diameter 2–3 mm; IV degrees: hyperemia diameter >3 mm.

Data analysis

The data were investigated for normality by Kolmogorov-Smirnov test. In vitro data were normal and analyzed by One-way ANOVA followed by LSD test while in vivo data were not normal and analyzed by Kruskal-wallis followed by Mann-Whitney test. Results are expressed as mean ± S.E.M. The accepted level of significance for all tests was $p < 0.05$.

Results and discussion

Physicochemical properties

The results for physicochemical properties of the prepared nanoliposomes are shown in Figure 1. The results did not show significant differences between nanoliposomes for size ($P>0.05$). The results showed a mean size of 150 nm for the prepared nanoliposomes. The results revealed that loading more rutin did not have any significant effects on size. The differences in size could be attributed to the composition of phospholipids, more significant polar phospholipid amounts, and higher compaction of the aqueous liposomal core [21]. Since the amount of rutin was less, it could not cause significant differences between groups.

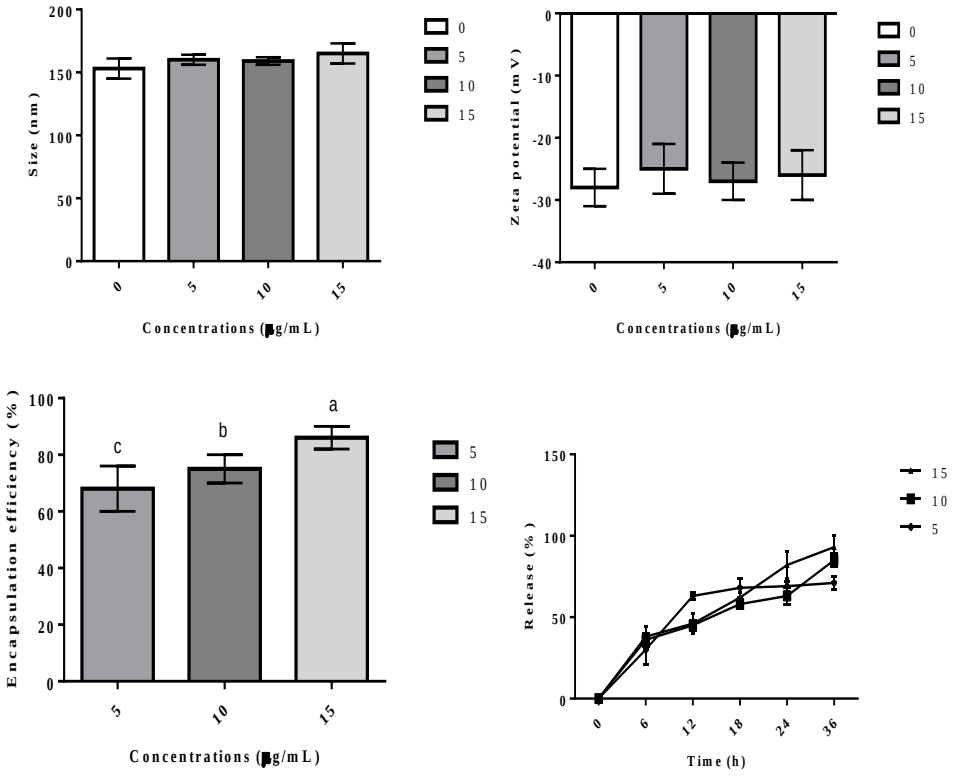

FIGURE 1 Physicochemical properties of the prepared nanoliposomes, the letters above figures show significant differences

The results for zeta potential did not show significant differences between groups. The values for zeta potential were negative. Negative potential could be attributed to a small portion of fatty acids ionized in the aqueous medium [21]. The results show that loading rutin did not significantly affect zeta potential.

The results showed the entrapment efficiency was increased with increasing rutin concentration. Seemingly, rutin fills open pores in nanoliposomes and increases their efficiency.

The results for release behavior of rutin showed that a significant part of rutin was released in the first hours for all the concentrations that could be attributed to electrostatic interactions between the positive charges of rutin and the negative charge of phosphate groups. Weak bonds between rutin and nanoliposomes may be a reason for increased release in the first hours. Nanoliposomes containing greater concentrations of rutin released a significant portion in a longer time.

Healing rate

The results for healing rate showed that the healing rate was 66.66% in the control group while the healing rate was 100% in the 15-R group. The results show that the addition of rutin into base ointments increased the healing rate. The results for wound healing activity agree with previous studies [22-24]. The increased rutin concentration could increase the healing rate attributed to a slower release of rutin and lesser degradation, as illustrated in the in vitro section. The healing activity of rutin could be attributed to its effects in decreasing inflammation, as will be discussed for IL-2, TNF-α, and edema and increased VEGF.

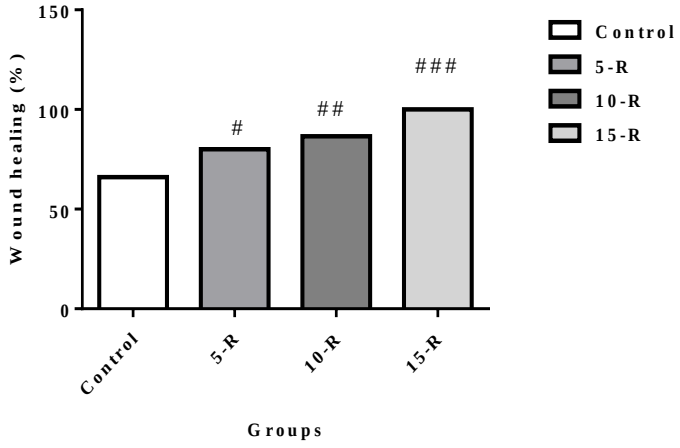

FIGURE 2 The healing rate in different groups. Superscripts (###, ##, and #) show significant differences between other groups with the control group in $p<0.001$, $p<0.01$, and $p<0.05$, respectively.

Results for Edema

The results for edema showed those greater edemas were found in control groups while increasing rutin decreased edema size. Edema is swelling caused by excess fluid trapped in tissues. Decreased edema in rutin groups could be attributed to the antibacterial and anti-inflammatory of rutin [24]. Greater concentrations are slowly released and show higher antibacterial activity.

The serum concentrations of TNF-α, IL-2, and VEGF

Figure 3 reveals the serum concentrations of TNF-α, IL-2 and VEGF. The results showed

that treating ulcers with ointments containing rutin, especially in higher concentrations, decreased the serum concentrations of TNF-α and IL-2 ad increased VEGF compared to the control group.

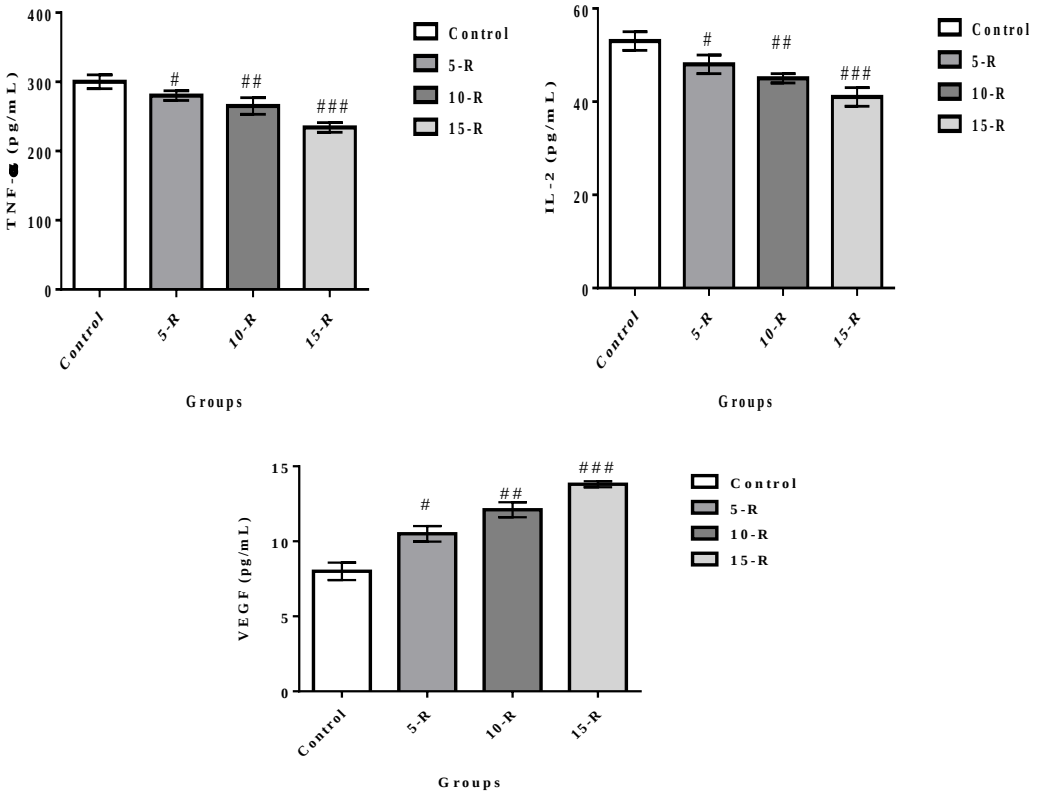

FIGURE 3 The results for the serum concentrations of TNF-α, IL-2, and VEGF in different groups. Superscripts (###, ##, and #) show significant differences between other groups with a control group in $p<0.001$, $p<0.01$, and $p<0.05$, respectively

TNFα starts the inflammation in the wound site [25] and is synthesized by several cells, acts as a pro-inflammatory cytokine, and delays the wound healing process [11]. It also increases vascular endothelial cells' permeability and activates lymphocyte and IL-2 [2]. Both IL-2 and TNF-α are involved in the inflammation phase and delay the healing process. Thus, rutin decreases inflammation by decreasing the serum concentrations of IL-2 and TNF-α. The results for inflammatory cytokines are in agreement with findings for edema. The ointments loaded with rutin also increased the serum concentrations of VEGF. VEGF is involved in the angiogenesis process and reduces tissue hypoxia and metabolic deficiencies [26]. In sum, rutin decreases inflammation and promotes the proliferation phase in oral ulcers.

Conclusion

In conclusion, we successfully prepared rutin nanoliposomes and added them into a base ointment. The ointments prepared from rutin decreased the inflammation and promoted the healing process in animal models. Rutin showed an excellent potential for the treatment of oral ulcers. It can be suggested to design a future on the effects of rutin in the healing process of oral ulcers.

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