Research Article

Preparation and evaluation of Luteolin gel for chronic wound healing

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Abstract

Objective: Wound healing is a complex dynamic process. Wound environment changes with the changing health status of the individual. Objective of present study was to prepare and evaluate luteolin gel for diabetic wound healing effect. **Material and methods:** Luteolin loaded hydrogel (1%w/w) was evaluated for wound healing activity using diabetic wound model. Healing effect was compared with Povidone iodine ointment. Healing effect was observed by contraction area and assessment of various biochemical parameters e.g. hydroxyproline measurement, protein estimation, and histological study. Results were compared with standard group and found significant greater than the control group. **Results and conclusion**: On day 18, Luteolin loaded hydrogel (1% w/w) and reference ointment treated group of animals were showed 100% and 90% wound contraction, respectively. On day 18 no scars were observed in animal treated with Luteolin loaded hydrogel (1% w/w) and reference ointment, which was an indication for complete healing. The hydroxyproline content in animals treated with Luteolin loaded hydrogel (1%w/w) and reference ointment was found significantly ($P< 0.05$) greater than control group of animals. In conclusion, Luteolin having strong antioxidant and anti-inflammatory potential as already reported by various researchers. These properties of luteolin may supported in present study of Luteolin loaded hydrogel for wound healing effect in diabetic condition.

Keywords: Wound healing, Luteolin, hydrogel, Povidone iodine ointment, diabetic wound

Introduction

The process by which tissue repair takes places is called as wound healing and is comprised of a continuous sequence of inflammation and repair, in which epithelial, endothelial, inflammatory cells, platelets and fibroblasts briefly come together outside their normal domains and interact to restore resemblances of their used discipline and having done so as to resume their normal function. Wound healing is a complex dynamic process. Wound environment changes with the changing heath status of the individual. The knowledge of the physiology of the normal wound healing trajectory through the phases of hemostasis, inflammation, granulation and maturation provides a framework for an understanding of the basic principles of wound healing (Kerstein, 1997).

Luteolin (3',4',5,7-tetrahydroxy flavone) is a flavone naturally occurring as a glycosylated form, and is present in different fruits and vegetables. Many researchers reported that luteolin possesses antioxidant, anticancer, antiinflammatory, and neuroprotective effects (Nabavi et al., 2015). Luteolin inhibited the LPS-induced release of TNF-α, IL-6, and NO by macrophages (Park et al., 2013). Luteolin significantly decreased the LPS-induced secretion of INF-γ, IL-6, COX-2, and iNOS in the alveolar macrophage and peripheral macrophage RAW 264.7 cell lines. This antiinflammatory action was accompanied by the suppression of NF-kappa B, IkB degradation, and AP-1 in LPS-activated macrophages, and it significantly blocked COX-2 expression in carrageenan-induced paw oedema (Zhang et al., 2017). Luteolin also suppresses inflammation in brain tissues and regulates different cell signalling pathways in neurodegenerative diseases and neuronal cell death (Nabavi et al., 2015). On the basis of previous research and literature, the present study was aimed to prepare gel formulation of luteolin for wound healing potential using diabetic wound model.

Material and Methods

Preparation of hydrogel formulations

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Five different hydrogel formulations with drug loaded and one control without drug was prepared according to the modified method of Chirayath et al., (2019). Briefly, different proportions of Carbopol 940 and Sodium CMC were dispersed in 50 ml of distilled water with continuous stirring. About 5 ml of distilled water was taken and required quantity of methyl paraben and propyl paraben were dissolved by heating on water bath and then Cooled. Glycerin was added to this solution. Accurately 1% w/v of Luteolin was taken to get optimized formulation and was added to the above swollen polymer under continuous stirring at 700 rpm in close vessel and maintaining the temperature 30°C until homogeneous gel was obtained and volume made up to 100 ml by adding remaining distilled water. At the end finally, required amount of 98% triethanolamine was added drop wise to the formulation for adjustment of required skin pH (6.8-7) and stirred slowly to mix uniformly (Table 1). The similar method was followed for preparation of control formulation without adding Luteolin.

Characterization of hydrogel formulation

Drug content determination

One gram of the prepared gel was mixed with 100ml of suitable solvent ethyl alcohol. Aliquots of different concentration were prepared by suitable dilutions after filtering the stock solution and the drug content was determined measuring the absorbance at 350 nm using UV/Vis spectrophotometer (Shimadzu UV 1700).

In-vitro drug release study

Franz diffusion cell with a diameter 3.9 cm was used in in-vitro release studies. A glass tube with both end open, 10 cm height and 3.9 cm outer diameter was used as a permeation cell. Accurately 1 gm sample was weighed and placed on a semipermeable cellophane membrane to occupy a circle of 3.9 cm diameter. The loaded membrane was stretched over the lower open end of a glass tube of 3.9 cm diameter and made water tight by rubber band. The tube (donor compartment) was immersed in a beaker containing 100 ml of phosphate buffer pH 6.8 (receptor compartment). The cell was immersed to a depth of 1 cm below the surface of buffer. The system temperature was maintained at 37°±1° and speed was maintained at 30 rpm throughout the experiment by magnetic stirrer. Samples 3 ml were withdrawn at intervals of 15, 30, 45, 60, 90, 120, 180, and 240 min, the volume of each sample was replaced by the same volume of fresh buffer to maintain constant volume. Samples were analyzed without dilution or filtration for Luteolin content spectrophotometrically at 350 nm (Gupta et al., 2007; Dutta et al., 2007). Calibration curve of luteolin was prepared of known concentrations in the appropriate range, to determine the amount of drug released.

Dermal irritation study of optimized formulation

A primary skin irritation test was conducted on rabbits to determine the potential of hydrogel to produce an irritation after a single topical application. Three healthy young adult albino rabbits of either sex were allows to free access to lab and fed commercial pellets diet with water ad libitum. Animals were acclimated to laboratories conditions for a period of 9 days prior to initiation of dosing. Animal room was kept at a constant temperature (19-24°C).

On the day before application, hairs of rabbits were removed from the dorsal and trunk area using a small animal clipper. On the day of dosing, but prior to application, the animals were examined for health and the skin checked for any abnormalities. No preexisting skin irritation was observed. 2-3 gms of the hydrogel was applied to 6 cm^2 intact area on each animal and caged. After 4 hr of exposure to hydrogel, the test sites were gently cleaned from any residual substance. Individual evaluation of test dose was scored according to Draize Scoring System at approximately 1, 24, 48 and 72 hr after removal of hydrogel (Draize et al., 1944).

Stability studies of prepared formulation

All prepared hydrogel formulations were subjected to a

Table 1: Formulations composition of hydrogel loaded with Luteolin

Ingredients	F1	F2	F3	F4	F5
Carbopol 940 (gm)	0.5	1	1.5	2	3
Sodium CMC	3	2	1	1	0.5
Luteolin (%w/w)	1	1	1	1	1
Propylene glycol 400 (5%)	5	5	5	5	5
Methyl Paraben (0.5%) (ml)	0.2	0.2	0.2	0.2	0.2
Propyl Paraben (0.2%) (ml)	5	5	5	5	5
Triethanolamine (ml)	q. s.	q. s.	q. s.	q. s.	q. s.
Distilled water (ml) q.s.	100	100	100	100	100

stability testing for six months as per ICH norms at a temperature and RH of 40°C ± 2°C/75% RH ± 5% RH respectively.

Stability of prepared hydrogel was evaluated in terms of physical changes, which would affect the stability and acceptability of the formulations.

Hydrogel formulations were evaluated in terms of physical changes like phase separation and color changes, odor, consistency of the formulations thereby affecting their stability and other desired formulation properties. Test samples of the hydrogel formulation were kept at different temperature conditions like at 40°C and room temperature for 30 days. Samples were periodically observed for physical changes like consistency and development of objectionable color and odor.

Wound healing activity of Luteolin loaded hydrogel

Animal protocol

For the incision, dead space, and diabetic wound models, Wistar albino rats (180-200 g) were selected. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and they were kept under standard environmental conditions of laboratory temperature and water *ad libitum*. The animals were maintain alternate cycle of darkness and light at 12 hours. They were acclimatized to the laboratory condition for 1 week before starting the experiment. The animals were fasted for at least 12 hours before the onset of experiment. The experimental protocols were approved by Institutional Animal Ethics Committee. Six animals were taken in each group and three groups were made in each wound model. Group I was denoted as Control group, and was treated with only simple gel base, while Group II denoted as treatment group, was treated with Luteolin loaded hydrogel (1%w/w), Group III was treated with standard Povidone Iodine Ointment USP (Zenith Drugs Pvt Ltd, India) ointment was used.

Diabetic wound creation

Elevated blood glucose levels may cause endothelial damage with potential occlusion of capillary vessels as well as hyperglycemia-induced leukocyte dysfunction, decreased chemotaxis and phagocytosis resulting in impaired wound healing and increased risk of infection. A model designed to study the combination of infection and advanced glycation end product-mediated cytokine up-regulation explains the increase in tissue destruction seen in diabetic periodontitis and how periodontal infection may complicate the severity of diabetes and the degree of metabolic control. Defective collagen metabolism in diabetics is also thought to be a factor in delayed wound healing. Hyperglycemia in animal studies is associated with increased collagenase and protease activity in rat gingiva and impaired vascular wound healing (Decker et al., 2005).

This model was selected to study of impaired and delayed healing in diabetic condition. Wistar albino rats (150-180 g) were made diabetic by being given a single injection of streptozotocin (STZ) prepared in Citrate buffer (0.1 M, pH 4.5) 50 mg/kg, i.p. after overnight fasting. Blood was taken from the orbital plexus, 24 h after the injection and glucose level was estimated using Glucometer. An excision wound was making on the rats showing elevated blood glucose (more than 250 mg/dl). Blood glucose level was estimate at the time of creation of the wounds and after treatment (Shukla et al., 1999).

Wound contraction and epithelization time

The percentage wound contraction was determined using the following formula:

$$\text{Percent wound contraction} = \frac{healed\ area}{total\ area} \times 100$$

Wound contraction was measured plannimetically using a transparent paper in each two days interval.

Biochemical estimation

The method of Woessner (1961) was used for the quantitative determination of hydroxyproline in biological material containing as little as one part of hydroxyproline in 4000 parts of amino acids. This method has been applied to a study of hydroxyproline distribution in cell particulates, tissue fluid and purified plant and animal proteins.

The measurement of protein in the tissue lysate was determined by treatment with a mixture of sodium tartrate, copper sulphate and sodium carbonate. This was left to stand for 10 minutes and then treated with Folin-Ciocalteau reagent that resulted in a bluish color in 20-30 minutes. The absorbance was measured in UV (Shimadzu, Japan) Spectrophotometer at 650 nm (Lowry et al., 1951).

Enzymatic and non-enzymatic antioxidant assay

From Diabetic wound, one part of granuloma tissue was used for antioxidant assay. Catalase was estimated following the breakdown of hydrogen peroxide according to the method of Beers and Sizer (1952). Superoxide dismutase (SOD) was assayed according to Misra and Fridovich (1972) based on the inhibition of epinephrine autoxidation by the enzyme. Reduced glutathione (GSH) content was determined in granuloma tissue by the method of Moron *et al*, 1979.

Histopathological study

Animals were anaesthetized before taking skin sample using diethyl ether. Wound tissue specimen from control, treated and reference group were collected and store in 10% formalin after that usual processing 6µm thick sections were cut and stained with haematoxylin and eosin (McManus and

Mowry, 1965). The histopathologic criteria were used with each animal for: epithelial proliferation, granulation tissue formation and organization, newly formed capillaries (identified by the presence of erythrocytes in their lumen) per site. Sections were qualitatively assessed under light microscope and were observed fibroblast proliferation, collagen maturation, angiogenesis and epithelialization.

Results and discussion

Carbopol-940 was used as gel former in order to select leading gelling agent. Preliminary, less than 1% of carbopol-940 with 1.3 ml of triethanolamine was tried for the hydrogel preparation (Table 1). In this concentration we found that, it forms very thin gel that liquefies within 6 h of preparation. Somewhat better gel was formed with greater than 1% of carbopol-940. Gel containing 1 to 3% of carbopol-940 with 1.3 ml tri-ethanolamine was tried, but we found that uniform and smooth gel were formed using carbopol-940 that did not liquefy upon keeping, for a long duration at room temperature.

Physical properties of prepared hydrogel such as color, appearance, homogeneity, consistency, phase separation and odor were observed. From the physical evaluation the color of the prepared gels was brownish in color and appearance of hydrogel was homogeneous and it was smooth on application. The hydrogel was found to be homogenous and good consistency and did not found any phase separation.

The pH value, viscosity and spreadability of the prepared hydrogels were studied at room temperature (Table 2). At initial phase, pH of prepared hydrogel was measured using pH meter (Systronics, India) at room temperature found ranging 6.4 to 6.6. Therefore, the pH of the gel having neutral value was desirable to skin since they did interfere with the physiology of the skin. The viscosity of gel was 123300-125500 cps was recorded at initial phase for hydrogel formulations containing Luteolin. Spreadability of both hydrogel formulations were recorded in the range of 13.85 to 15.23 g.cm/s.

The drug content was determines by spectrophotometric method and found as 0.085µg/ml. This content was also basis for drug release study of hydrogel formulation. Percentage drug release of hydrogel was observed initially to be 10.25% (at 15 min.) and 52.84% (at 240 min.) for F1 formulation. All other formulations F2, F3, F4 and F5 formulations (Table 3). Out of all different formulations, F2 formulation was showing highest percentage drug release at 240 min as 75.47, as compared to other formulations. These results showing that the hydrogel formulations containing 1.0% Carbopol 940 and 2% of Sodium CMC having higher percentage of Luteolin release in 240 min. All other combinations of hydrogel were showing lower percentage of Luteolin release up to 240 min.

Results of all other evaluation parameters e.g. pH, viscosity, spredability and consistency were found suitable in F2 hydrogel formulation combination. So the optimize

Table 2. Evaluation of prepared Luteolin loaded hydrogel formulations

Parameters	F1	F2	F3	F4	F5
pH	6.4	6.5	6.6	6.5	6.6
Viscosity (cps)	123300	124100	125000	1248500	125500
Spreadability (g.cm/s)	15.24±0.08	13.85±0.14	15.23±0.28	15.20±0.37	14.75±0.87

Table 3. Percentage drug release of prepared luteolin loaded hydrogel

Time interval (min)	% drug release of formulations				
	F1	F2	F3	F4	F5
15	10.25	18.75	12.77	14.20	10.44
30	12.62	23.65	17.66	18.33	13.62
45	17.45	28.53	21.43	24.20	18.62
60	21.95	36.62	34.27	31.74	23.43
90	30.42	42.66	41.25	44.26	29.67
120	39.52	55.75	49.55	49.36	33.42
180	42.12	64.42	53.64	51.23	43.75
240	52.84	75.47	61.22	55.75	50.10

composition of F2 formulation was observed as 1.0 g of Carbopol 940 and 2% of Sodium CMC for best drug release profile.

The skin irritation study was performed on rabbits and so as to assess gentleness of the prepared formulations against the skin irritation. The optimized formulation, F2 was applied on the rabbit skin and its adverse effects such as change in color and swelling of the skin were noted by visual observation.

The stability study of all prepared hydrogel was performed according to ICH guideline (2013) by keeping at 27±1ºC for about thirty days and again physical properties were observed. After thirty days it was observed that all properties were same except color. The color of the gel was little faint bluish. There was no phase separation and liquefaction of the gel in the period of thirty days. Other parameters were evaluated after thirty days.

The spreadability of formulation depends on its viscosity. The spreadability of formulations was recorded for optimized gels. These observations have indicated that the gel was easily spreadable in response to the little force applied. These assured that the formulation could maintain a good wet contact time when applied at the target site (Deuschle et al., 2015).

The pH of the prepared formulations was found almost near to the pH of skin. Prepared hydrogel was found to be stable even at room temperatures, and no any separation of oil phase was observed at elevated temperature, 40^{0}C±2 °C.

There was no evidence of phase separation, development of objectionable odour or any other evidence of physical instability and effect on storage at varying temperature of hydrogel.

The objective of this study was to determine the potential of formulations to produce irritation from a single topical application to the skin of rabbits. All animals were free from dermal irritation after 48 h. Apart from the dermal irritation noted, all animals appeared active and healthy and with no other signs of unpleasant toxicity or abnormal behavior.

Luteolin loaded hydrogel (1%w/w) was evaluated for wound healing activity using diabetic wound model. Healing effect was compared with Povidone iodine ointment. Healing effect was observed by contraction area and assessment of various biochemical parameters e.g. hydroxyproline measurement, protein estimation, and histological study. Results were compared with standard group and found significant greater than the control group.

The wound contraction percentage was determined at two days interval. The wound margins were traced and measured to calculate the non healed area which was then subtracted from the original wound area to obtain the healed area (Figure 1). The wound contraction percentage was determined from the first time on second day after application of different formulations and reference ointment. This was carryout at

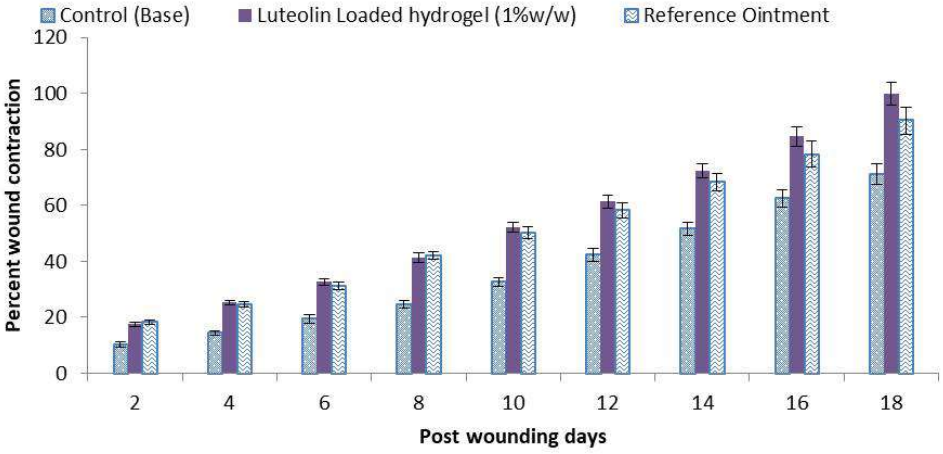

Figure 1. Effect of Luteolin loaded hydrogel on percent wound contraction of diabetic wound in rats

Table 4. Effect of Luteolin loaded hydrogel on hydroxyproline and protein content of tissues from diabetic wound in rats

Groups	Hydroxyproline (mg/ g tissue)	Protein content (mg/g tissue)
Control (gel Base)	27.65±1.42	34.29±1.86
Luteolin loaded hydrogel (1%w/w)	54.48±2.64*	72.55±3.82*
Reference Ointment	51.26±2.94*	69.24±3.41*

n = 6 albino rats per group, value represents Mean S.D. *P>0.05, when compared each treated group with control group

Table 5. Effect of Luteolin loaded hydrogel on enzymatic and non-enzymatic antioxidant level of tissues from diabetic wound in rats

Groups	Enzymatic and non-enzymatic assay		
	SOD(µg/50mg tissue)	CAT(µmol/50 mg tissue)	GSH(µmol/50 mg tissue)
Control (Base)	41.28±1.75	30.27±1.08	19.34±0.85
Luteolin loaded hydrogel (1%w/w)	76.85±3.61*	68.37±3.17*	42.08±1.76*
Reference Ointment	75.24±3.28*	65.72±2.98*	41.75±1.45*

n = 6 albino rats per group, value represents Mean S.D. *P>0.05, when compared each treated group with control group

two days intervals for duration of three weeks. In the treatment group with EETIS Ointment (2.5 and 5%w/w), and reference ointment group of animals on days 2 not observed significant changes in percent contraction. After day 4 to 20 the group treated with Luteolin loaded hydrogel and reference ointment was exhibited significant increase in the percent wound contractions as compared to control group of animals. From 10 to 20 days, in group treatment with Luteolin loaded hydrogel was observed significant difference in percentage of wound contraction when compared to control group, and reference group of animals.

On day 18, Luteolin loaded hydrogel (1% w/w) and reference ointment treated group of animals were showed 100% and 90% wound contraction, respectively. On day 18 no scars were observed in animal treated with Luteolin loaded hydrogel (1% w/w) and reference ointment, which was an indication for complete healing.

The hydroxyproline content in the wound tissue of the treated with Luteolin loaded hydrogel and control group of animals are shown in table 4. The hydroxyproline content in animals treated with Luteolin loaded hydrogel (1%w/w) (54.48±2.64) and reference ointment (51.26±2.94) was found significantly (P< 0.05) greater than control (27.65±1.42) group of animals.

Over the 20-day treatment period, the protein content of wounds treatment groups with 27.65±1.42 (1%w/w) (72.55±3.82) and reference ointment (69.24±3.41) was found significantly (P< 0.05) increased when compared to control (34.29±1.86) group of animals.

The enzymatic and non-enzymatic assay during wound healing in skin tissues are given in table 5. The activity of antioxidant enzymes was increased in Luteolin loaded hydrogel (1%w/w) treated animals when compared with reference group. Due to increased activity of antioxidant enzymes, their content was lower during healing process.

Histopathological study was showed that well organized collagen fibers, increase in fibroblast cells and new blood vessels formation was observed in treated groups of Luteolin loaded hydrogel and reference ointment), when compared to control group. In the control group ointment group of animals was found a more marked proliferation of angioblasts and fibroblast with infilteration of large number of lymphocytes and microphages and a few neotrophils on 20 days.

In the group treated with Luteolin loaded hydrogel, formation of dense fibrous tissue and blood capillaries was observed on 20 days. The arrangement of capillaries was perpendicular to the fibrous tissue. Mature connective tissue and a few capillaries with a thick epidermal lining at the periphery of the wound were observed at 20 day. The differences in severity of vascular and cellular changes between the wounds treated with various medicaments might be due to differences in chemical constituents and physical properties of various medicaments.

Conclusion

The results indicate they were nearly same in the terms of applicability or spreading capacity. Storage even at accelerated stability conditions does not influence the stability of prepared hydrogel. Thus it may be concluded that formulations were adequate and satisfactory as far as physical parameters are concerned. In conclusion, Luteolin having strong antioxidant and anti-inflammatory potential as already reported by various researchers. These properties of luteolin may supported in present study of Luteolin loaded hydrogel for wound healing effect in diabetic condition.

Conflict of interest: None

References

Anonymous, British Pharmacopoeia, General Medical Council, The Pharmaceutical Press, 17 Bloomsbury Square, London, W CI.P 1953.

Beers RF, Sizer IW. 1952. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. Journal of Biological Chemistry 195, 133–140.

Chirayath RB, Jayakumar R, Biswas R, Vijayachandran LS. Development of Mangifera indica

leaf extract incorporated carbopol hydrogel and its antibacterial efficacy against Staphylococcus aureus. Colloids Surf B Biointerfaces 2019;178:377-84.

Decker RT, Sirois DA, Mobley CC. Nutrition and oral medicine, Winkler MF, Makowski S, Wound Healing, Humana Press Totowa, New Jersey, 2005, pp 273-283.

Deuschle VC, Deuschle RA, Bortoluzzi MR, Athayde ML. 2015. Physical chemistry evaluation of stability, spreadability, in vitro antioxidant, and photo-protective capacities of topical formulations containing Calendula officinalis L. leaf extract. Brazilian Journal of Pharmaceutical Sciences 51:63-75.

Draize JH, Woodard G, Calvery HO. 1944. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. Journal of Pharmacology and Experimental Therapeutics 82:377-90.

Dutta D, Devi SS, Krishnamurthi K, Kumar K, Vyas P, Muthal PL. 2007. Modulatory effect of distillate of Ocimum sanctum leaf extract (Tulsi) on human lymphocytes against genotoxicants. Biomedical and Environmental Sciences 20:226-34.

Gupta P, Yadav DK, Siripurapu KB, Palit G, Maurya R. 2007. Constituents of Ocimum sanctum with antistress activity. Journal of Natural Products 70:1410-6.

Kerstein MD. 1997. The scientific basis of healing. Advance Wound Care 10(3): 30-36.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. 1951. Protein measurement with the Folin Phenol Reagent. Journal of Biological Chemistry 193: 265-275.

MCmanus, J. F. A., Mowry, R. W. Staining Methods, histologic and histochemical. Harper 7 Row New York, Evanston, London, 1965.

Misra, H.P., Fridovich, I., 1972. The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. Journal of Biological Chemistry 247, 3170–3175.

Moron, M.A., Depierre, J.W., Mannervick, B., 1979. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochimica et Biophysica Acta 582(1), 67–78.

Nabavi S.F., Braidy N., Gortzi O., Sobarzo-Sanchez E., Daglia M., Skalicka-Wozniak K., Nabavi S.M. Luteolin as an anti-inflammatory and neuroprotective agent: A brief review. Brain Research Bulletin 2015;119:1–11.

Shukla, A., Rasik, A.M., Dhawan, B.N. Asiaticoside induced elevation of antioxidant level in healing wounds. Phytotherapy Research 1999;13, 50-54.

Woessner, J. F. The determination of hydroxyproline in tissue and protein samples containing small portion of this imino acid. Archives of Biochemistry and Biophysics 1961; 193, 440-447.

Zhang X, Du Q, Yang Y, Wang J, Dou S, Liu C, et al. The protective effect of Luteolin on myocardial ischemia/reperfusion (I/R) injury through TLR4/NFκB/NLRP3 inflammasome pathway. Biomedicine & Pharmacotherapy 2017; 91:1042-1052.