The effect of rose hip on experimental anti-GBM glomerulonephritis in systemic lupus erythematosus murine models

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Submission Date: December 1st, 2021; Acceptance Date: December 22nd, 2021; Publication Date: December 24th, 2021

Please cite this article as: Martirosyan D., Min S.Y., Xie C., Yan M., Bashmakov A., Williams S., Mohan C. The effect of rose hip on experimental anti-GBM glomerulonephritis in systemic lupus erythematosus murine models. Functional Food Science 2021; 1(12): 86-96. DOI: https://www.doi.org/10.31989/ffs.v1i12.873

ABSTRACT

Background: Systemic lupus erythematosus (SLE or lupus) is a chronic autoimmune disease with ominous end organ manifestations significantly affecting the kidneys and joints. One of the most frequent manifestations is glomerulonephritis (GN), a renal disease distinguished by inflammation of the glomeruli that often leads to end stage kidney failure. Treatments often have severe side effects. Rose hip (RH) is derived from Rosa canina L. and has been used as a medicinal plant for centuries; it contains numerous beneficial constituents, and has the capacity to counter lipid peroxidation, oxidative stress, and inflammation.

Methods: Nephritis was induced in 129/svJ strain mice using anti-glomerular basement membrane antibodies (anti-GBM). The experimental group was fed whole RH preparation (100mg/kg body weight per day) by oral gavage from D5 to D10; the control group was fed the diluent used to dissolve RH (10 mice per group). Mice were sacrificed on D11 and phenotyped for disease. Blood urea nitrogen (BUN) and proteinuria were measured; flow cytometry of kidneys was performed on both groups.
**Introduction**

Systemic lupus erythematosus (SLE or lupus) is a chronic autoimmune disease with ominous end organ manifestations affecting the kidneys and joints. One of the most frequent manifestations of SLE is glomerulonephritis (GN) [1]. GN is a renal disease (usually of both kidneys) distinguished by inflammation of the glomeruli, or small blood vessels in the kidneys, that often leads to end stage kidney failure [2]. It is characterized by proteinuria, hematuria, edema and hypertension followed by the increase of blood urea nitrogen (BUN), which is one of the major indicators of kidney function [3-4]. The occasionally seen immunosuppressive treatment currently used with corticosteroids and cytotoxic agents is often complicated by severe side effects. Lupus nephritis is a form of GN often found in approximately 50-60% of SLE patients [5]. Experimental anti-GBM (anti-glomerular basement membrane antibody) induced nephritis models downstream pathogenic cascades that mediate disease in spontaneous lupus nephritis, since both the induced and spontaneous models share molecular pathways that

**Results:** RH treatment decreased proteinuria, blood urea nitrogen, CD4+, CD8+, CD11b’Gr-1+ (neutrophil), and CD11b’CD11c+ (myeloid cells) compared with nephritis control. The presence of vitamin C was confirmed. RH largely maintained its total antioxidant capacity and some vitamin C content for 24 hours, as well as at least 7 days after preparation.

**Conclusion:** Our preliminary results confirmed that RH has antioxidative properties, significant anti-inflammatory effects, and may be useful in managing glomerulonephritis

**Keywords:** Rose hip, glomerulonephritis, systemic lupus erythematosus, lupus, proteinuria, blood urea nitrogen, CD4+, CD8+, CD11b’Gr-1+ (neutrophil), and CD11b’CD11c+ (myeloid cells)

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lead to disease [6-7]. Anti-glomerular basement membrane antibody-induced glomerulonephritis (anti-GBM-GN) is the most serious form of immune-mediated GN [8].

Rose hip (RH) is derived from Rosa canina L., which has been used as a medicinal plant for over 2000 years [9]. It is rich in vitamin C (10-50 times higher than the levels in oranges), vitamin A, contains anti-inflammatory galactolipids, linoleic acid, α-linolenic acid, vitamin E, the B vitamins riboflavin and folate, as well as large amounts of polyphenols, bioflavonoids and carotenoids including beta-carotene, lycopene, beta-choytoxanthin, rubixanthin, zeaxanthin and lutein [9-21]. One galactolipid of interest is (2S)-1,2-di-O-[(9Z,12Z,15Z)-octadeca-9,12,15-trienoyl]-3-O-8-D-galactopyranosyl glycerol (GOPO), which was shown to possess anti-inflammatory effects [22]. In Figure 1, we present rose hip nutritional facts, as provided by Starwest Botanicals Inc. Notably, it has the capacity to counter lipid peroxidation, oxidative stress and inflammation [16-17, 23-24]. RH has previously been tested for its efficacy in ameliorating arthritis – both rheumatoid arthritis and osteoarthritis, with encouraging results [25-28]. A number of studies have also tested the anti-inflammatory and analgesic effects of RH in animal models or in cell cultures [16-17, 23-24, 29-32]. In randomized double blind placebo controlled studies, RH powder was also shown to reduce osteoarthritis symptoms [14, 25, 27, 32-35]. Pretreatment with a single dose of RH produced significant dose-dependent anti-inflammatory effects on carrageenin-induced rat hind paw edema, as well as xylene-induced mouse ear edema. Past studies found the RH mechanism of action to involve neutralizing reactive oxygen species, down-regulating cyclooxygenase (COX)-2 expression, reducing NO production through inhibiting iNOS, reducing C-reactive protein levels, countering oxidative stress and reducing lipid peroxidation [13, 16-17, 23-24, 30]. The impact of RH on lupus and nephritis has not been examined previously.

![Figure 1. Nutritional facts of rose hips, provided by Starwest Botanicals Inc.](https://example.com/rosehip_nutrition.png)
In the present study, we also examine total antioxidant capacity, and vitamin C (ascorbic acid) content of RH. In addition to the initial quantification, total antioxidant capacity and vitamin C content will be measured both after 24 hours and 7 days, which have not previously been reported. High-performance liquid chromatography (HPLC) is a technique that uses column separation for the high resolution separation and determination of analytes [36-38]. HPLC can be detected via ultraviolet (UV) absorbance (HPLC-UV) where the analyte’s maximum UV absorption wavelength must match that of the standard material within 2 nm for identification [36, 39]. Similarly, ultraviolet-visible (UV-VIS) spectroscopy can be used to measure the absorbance spectra of a substance, yielding qualitative and quantitative information for identification [40].

Currently, RH is consumed in yogurts, soups, probiotic drinks, marmalade, fruit juice, and tea [14], but more RH based products possibly optimized for disease treatment could be advantageous. The long-term goal of this study is to lay the groundwork for incorporating RH into common foods consumed by the public in order to provide a potentially cost effective, novel functional food product, specifically designed for the management of chronic autoimmune diseases such as lupus. The prospective innovation in the management of chronic autoimmunity may also significantly impact other chronic diseases marked by inflammation and oxidative stress.

METHODS

**Obtaining RH and Quality Control:** RH was obtained from Starwest Botanicals Inc (Rancho Cordova, CA). Raw RH material was inspected upon arrival to verify the correct species and part of the plant. Prior to processing, it was sorted and washed. Afterwards, it was sampled and inspected to ensure that they met our quality control criteria.

**Preparing RH extract:** 100 ml of boiling water was poured on 5 g of plant material. The mixture was left to stand for 30 minutes at a steady temperature during the time of infusion. After final filtration, the RH extract was allowed to cool and ready for use in experiments. The final extract was stored in a refrigerator at 4-6°C.

**Quantifying vitamin C, and total antioxidant capacity in RH:** RH was sent to Interek Champaign Laboratories for vitamin C (ascorbic acid), and total antioxidant capacity analysis. High-performance liquid chromatography with ultraviolet detection (HPLC–UV) was used for measuring vitamin C [41], while ultraviolet-visible spectroscopy (UV-VIS) was used for total antioxidant capacity [42].

**RH treatment:** Male 8-week-old 129/svJ mice were administered rabbit anti-mouse-GBM sera in the adjuvant on day -5 (5 days before induction of anti-GBM-GN) for pre-sensitization, as detailed by Peng and co-authors [8]. On day 0, the mice received anti-GBM serum; 200 μg of total IgG in a 300 μl volume was administered intravenously per mouse and subsequently divided 5 times into RH (100mg/kg, orally) and vehicle-treated groups. The mice were fed whole RH preparation (100mg/kg body weight per day) by oral gavage, from D5 to D10 (10 mice per group). The control mice were fed
the diluent used to dissolve RH. The mice were sacrificed on D11 and phenotyped for disease. Mice were followed for BUN from serum.

**Flow Cytometry**: Flow cytometry of kidneys was performed on both RH and vehicle-treated groups, as outlined by Min et al [43]. A lysis buffer consisting of 10 mM potassium bicarbonate (KHCO3), 0.15 M ammonium chloride (NH4Cl) and 0.1 M ethylenediaminetetraacetic acid (EDTA), pH 7.2, was used to deplete red blood cells and lymph node cells from splenocytes. After preparing single cell suspensions, flow cytometry was conducted with FACSCalibur (BD Biosciences, San Jose, CA) and BD CellQuest Pro Software (BD Biosciences). Afterwards, FloJo Software (FlowJo, LLC, Ashland, OR) was used to analyze the data.

**RESULTS**

**Vitamin C, and total antioxidant capacity**: As shown in Table 1, 1 g of RH was found to contain 0.78 mg of vitamin C (ascorbic acid) during the initial analysis. After 24 hours, the vitamin C content decreased to 0.67 mg, and continued decreasing to 0.35 mg after 7 days. The total antioxidant capacity was 238 U/g initially, dropped to 216 U/G after 24 hours, then increased to 259 U/g after 7 days.

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<th>Analysis</th>
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<tr>
<td>Vitamin C (Ascorbic Acid)</td>
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<td>Initial</td>
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<td>Total antioxidant capacity</td>
<td>UV-VIS</td>
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<td>238 U/g</td>
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**BUN, Proteinuria, FACS**: The administration of RH significantly reduced the development of proteinuria and BUN at day 11 after initiation of anti-GBM GN, when compared with animals that received vehicle-treated glomerulonephritis mice. The kidney FACS images similarly show that mice given RH had significantly CD4+ and CD8+ T cell infiltration 11 days after anti-GBM injection compared with vehicle treated mice. Quantitative analysis verified that the percentage of CD11b(high)Gr-1(high) monocytes had increased in vehicle-treated nephritis mice kidneys, whereas that of RH-treated mice was significantly decreased in infiltrated kidneys. Shown are the proteinuria and BUN levels (Figure 2A and 2B) in the 2 groups of mice on D0 and D11, and representative flow cytometric plots (Figures 2C and 2D) depicting the numbers of T-cells and myeloid cells within the kidneys of these mice.
Functional Food Science 2021; 1(12): 86-96

DISCUSSION

This study examined the anti-inflammatory and antioxidant properties of rose hip (*Rosa canina* L.), and its effect on anti-GBM induced glomerulonephritis. While the control-fed mice exhibited significant renal disease, the RH-fed mice exhibited reduced proteinuria and BUN. Flow cytometric analysis of the renal infiltrates indicated significantly reduced numbers of CD4+ and CD8+ T-cells, as well as CD11b+Gr-1+ (neutrophil), and CD11b+CD11c+ (myeloid cells) compared with vehicle control-fed mice.

The main function of CD8+ T cells is the defense against intracellular pathogens and tumors, while CD4+ T cells activate and suppress immune reaction [44]. GN may be related to the nature of the nephritogenic immune response. T cells, notably CD4+ and CD8+, appear to be instrumental in developing most forms of GN, initiating, directing, and amplifying immune responses [2, 45]. Inflammatory cell infiltration in the renal interstitium is crucial to developing lupus nephritis; both CD4+ and CD8+ T cells are main components in the infiltrate, though macrophages, B cells, and plasma cells are also present.
Moreover, CD11b helps regulate innate immune cell adhesion, migration, and phagocytosis [5]. The CD11b\(^+\) cells expressed high levels of Gr1, a marker of proinflammatory monocytes, which can give rise to both dendritic cells (DCs) and macrophages. Considering RH is known to have anti-inflammatory properties, the decreased CD4\(^+\) and CD8\(^+\) T-cell, CD11b\(^+\)Gr-1\(^+\), and CD11b\(^+\)CD11c\(^+\) cell numbers in the experimental group are not too surprising.

HPLC-UV analysis confirmed the presence of vitamin C, while UV-VIS affirmed RH’s antioxidative properties. Ghendov-Mosanu and coworkers obtained 3.1 mg/100 g (or 0.031 mg/1 g) cis-lycopene and all-trans-lycopene [20]. Mihaylova et al measured 113.23 μg/g (or 0.11323 mg/1 g), while Razungles and co-authors observed 0.111 mg/g [19, 47-48]. Moreover, in the present study, the vitamin C content was found to be 0.78 mg initially, 0.67 mg after 24 hours, then 0.35 mg after 7 days. Though comparable to Fascella et al’s 513.9 mg/100 g (or 0.5139 mg/1 g) [18], the obtained amounts are all higher than what most studies reported for R. canina [19, 49-51]. A similar UV-VIS total antioxidant capacity analysis was not found, however, numerous studies note RH’s antioxidant capacity, constituents, and activities [9-10, 13-14, 18, 21, 47]. Since RH largely maintains its total antioxidant capacity and some vitamin C content for 24 hours, as well as at least 7 days after preparation, it has great potential as a functional food.

Vitamin A deficiency has previously been shown to decrease immune cells, namely CD4\(^+\)CD25\(^+\) T cells and CD8\(^+\) intestinal intraepithelial lymphocytes [52]. Since RH is rich in vitamin A, it is possible that the observed immunosuppressive effects are related. Moreover, rose hip powder and GOPO is known to attenuate inflammatory responses and down regulate catabolic processes in osteoarthritis or rheumatoid arthritis [35, 53]. Galactolipids, notably GOPO itself, have also demonstrated anti-inflammatory activity [15, 22, 54-55]. Jäger et al found that GOPO is not the only anti-inflammatory fatty acid, since COX inhibitory activity was still present after the galactolipid was extracted [9, 16]. In a following study, Jäger and coworkers found that linoleic acid and α-linolenic acid in RH also inhibit COX-1 and COX-2 [9, 17]. The other RH components, such as vitamin E, the B vitamins riboflavin and folate, polyphenols, bioflavonoids and carotenoids, may also play a role. The phenolic and flavonoid composition of RH correlates with antioxidant activity [54, 56]. Even excluding vitamin C, antioxidant activity was still observed [23, 54].

As far as other similar studies, Lattanzio et al also utilized a 100 mg/kg RH dose and observed anti-inflammatory, as well as antioxidative, activity in rats with carrageenin-induced edema [30]. Winther and coworkers found that daily consumption of rose hip powder reduced chemotaxis of peripheral blood neutrophils [57]. Similarly, Karazmi and Winther found that RH decreased peripheral blood polymorphonuclear leucocytes, and serum creatinine and C-reactive protein [58].

A few studies examined potential functional foods that reduce renal inflammation or nephritis. Green tea polyphenol (−)-epigallocatechin-3-gallate was shown to decrease proteinuria in anti-GBM-GN by Ye and coworkers [59]. Martirosyan et al reported amaranth oil may decrease proteinuria in lupus prone mice [60]. Chen and co-authors found that “kidney tea” (Clerodendranthus spicatus) compounds decreased pro-inflammatory cytokines in liposaccharide induced renal epithelia cells [61]. Wang et al observed that Dendrobium candidum treated mice had decreased BUN, urine protein, as well as cytokine levels [62]. Jiang and
coworkers utilized polysaccharide of large yellow croaker swim bladder and obtained similar results [63].

To our knowledge, RH has not previously been examined as a potential treatment and functional food for glomerulonephritis. That being said, past researchers found that a hot water RH extract displayed an anti-prediabetic effect and could be a potential functional food [64]. Overall, RH is a promising functional food candidate. To completely satisfy the Functional Food Center’s definition of a functional food though, the exact dosages for specific health benefits should be detailed in further studies [65-67]. Future studies should also examine the efficacy of RH in experimentally induced target organ inflammation and spontaneously arising chronic autoimmunity; this could pave the way towards the production of functional foods on the premise of RH for human consumption.

CONCLUSION
Our preliminary results confirmed that rose hip has antioxidative properties, significant anti-inflammatory effects, and may be useful in managing glomerulonephritis. This data can be used for the creation of new functional food products for the management of glomerulonephritis.

Abbreviations: Anti-GBM: anti-glomerular basement membrane antibody; Anti-GBM-GN: anti-glomerular basement membrane antibody-induced glomerulonephritis; DCs: dendritic cells; GOPO: (2S)-1,2-di-O-[(9Z,12Z,15Z)-octadeca-9,12,15-trienoyl]-3-O-ß-D-galactopyranosyl glycerol, GN: glomerulonephritis; HPLC: high-performance liquid chromatography; SLE: systemic lupus erythematosus; RH: rose hip; UV: ultraviolet; UV-VIS: ultraviolet-visible spectroscopy

Conflicts of Interest: There are no conflicts of interest associated with this study.

Authors’ contribution: The original idea (usage of RH extract as an anti-GBM glomerulonephritis in systemic lupus erythematosus) was conceived by DM and was discussed with CM. The main focus and ideas of the experiments finally agreed with SM. The experiments were conducted and analyzed by AB, CX, MY, DM. Experimental data was analyzed by SM, CM, CX, MY, and discussed with DM and CM. The main text of the paper including methods were written by SM, SW and DM. The manuscript was revised, edited, and formatted by DM and SW

Acknowledgement: We would like to thank the Department of Internal Medicine at The University of Texas Southwestern Medical Center for their support, making this scientific investigation possible.

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