Research Article **Open Access**

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

Danik Martirosyan[1,2*], So-Youn Min[1], Chun Xie[1], Mei Yan[1,] Anna Bashmakov[1], Samantha Williams[2], Chandra Mohan[1]

[1]Division of Rheumatology, Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, Texas, USA; [2]Functional Food Center/Functional Food Institute, Dallas, TX, USA

***Corresponding Author:** Danik Martirosyan, PhD, Functional Food Center/Functional Food Institute, Dallas, TX, USA

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

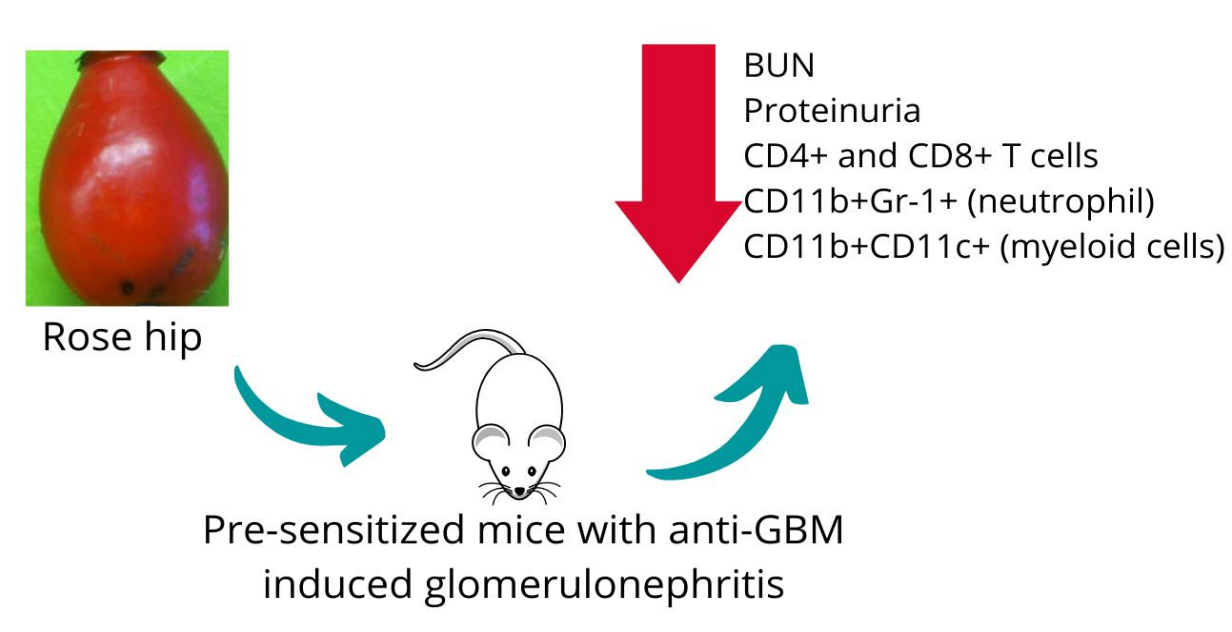
Background: Systemic lupus erythematosus (SLE or lupus) is a chronic autoimmune disease with ominous end organ manifestations significantly affecting the kidneys and joints. One of the most frequent manifestations is glomerulonephritis (GN), a renal disease distinguished by inflammation of the glomeruli 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.

Results: RH treatment decreased proteinuria, blood urea nitrogen, $CD4^+$, $CD8^+$, $CD11b^+Gr\text{-}1^+$ (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, $CD4^+$, $CD8^+$, $CD11b^+Gr\text{-}1^+$ (neutrophil), and $CD11b^+CD11c^+$ (myeloid cells)



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

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-chryptoxanthin, 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-ß-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.

NUTRITIONAL INFORMATION

PRODUCT NAME: **ROSEHIPS**
BOTANICAL NAME: *ROSA CANINA*

Nutrition Facts

Serving Size: (100 grams)

Amount Per Serving		
Calories:	679	
		% Daily Value*
Total Fat	0.6 g	1%
Saturated Fat	0 g	0%
Cholesterol	0 mg	0%
Sodium	7.3 mg	0%
Total Carbohydrates	70 g	23%
Dietary Fiber	44 g	176%
Sugars	5 g	~
Protein	3 g	5%

Vitamin A	133%	Vitamin C	1040%
Iron	11%	Calcium	31%

*Percent Daily Values are based on a 2000 calorie diet. Your daily values may be higher or lower depending on your calorie needs

Figure 1. Nutritional facts of rose hips, provided by Starwest Botanicals Inc.

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

Table 1. Vitamin C (ascorbic acid), and total antioxidant capacity of 1g RH. HPLC-UV was used to measure vitamin C (ascorbic acid) content, while UV-VIS was utilized for total antioxidant capacity. Analyses were run upon initial receipt, as well as after 24 hours and after 7 days.

Analysis	Method	Timeframe	Results
Vitamin C (Ascorbic Acid)	HPLC-UV	Initial	0.78 mg
		24 hours	0.67 mg
		7 days	0.35 mg
Total antioxidant capacity	UV-VIS	Initial	238 U/g
		24 hours	216 U/g
		7 days	259 U/g

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 $CD11b^{hi}Gr\text{-}1^{hi}$ 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.

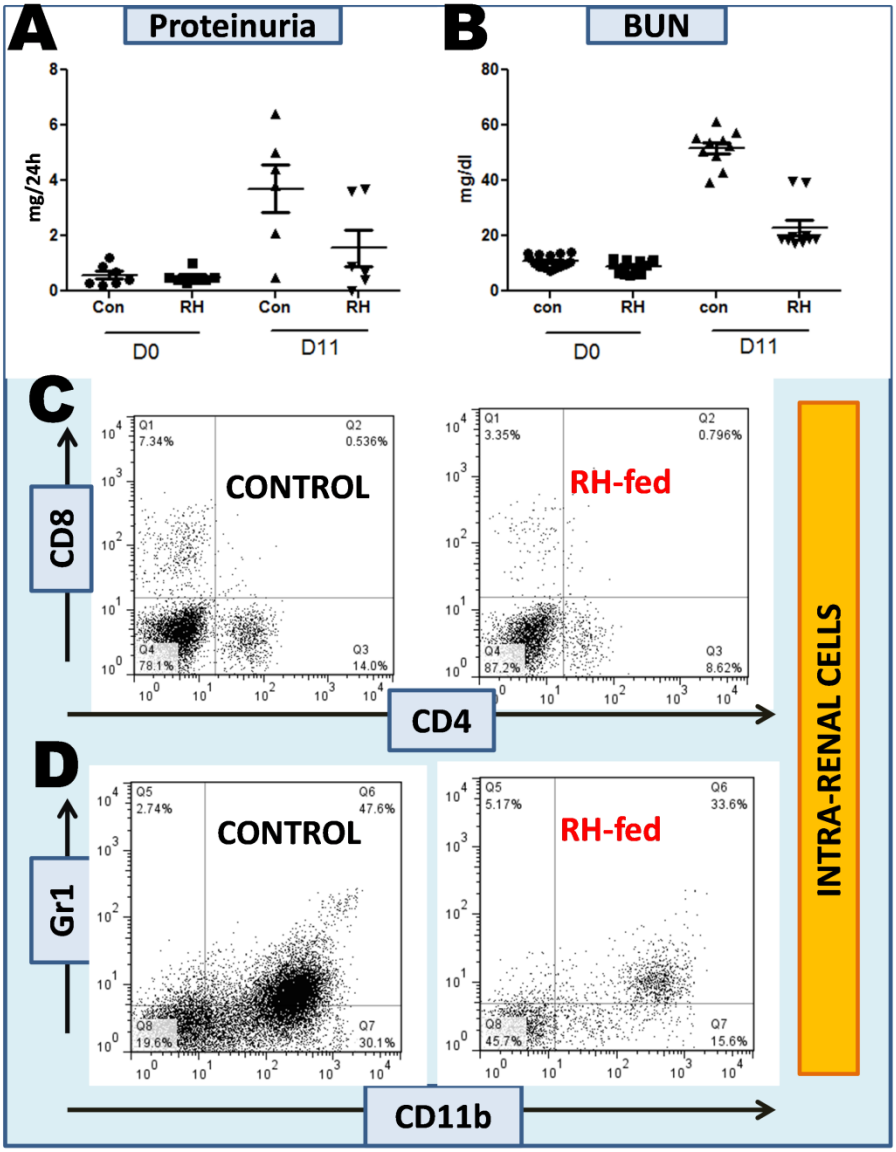


Figure 2. Proteinuria, BUN, and representative flow cytometry results for RH fed and control groups.

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 $CD11b^+Gr\text{-}1^+$ (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

[46]. Moreover, CD11b helps regulate innate immune cell adhesion, migration, and phagocytosis [5]. The $CD11b^{hi}$ 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, $CD11b^+Gr\text{-}1^+$, 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 0.111mg/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.

REFERENCES

1. Mok CC. Understanding lupus nephritis: diagnosis, management, and treatment options. Int J Womens Health 2012, 4:213-222. https://www.doi.org/10.2147/IJWH.S28034
2. Chadban S, Atkins R. Glomerulonephritis. The Lancet 2005, 365(9473):1797-1806. https://www.doi.org/10.1016/S0140-6736(05)66583-X
3. Khanna R. Clinical presentation & management of glomerular diseases: hematuria, nephritic & nephrotic syndrome. Mo Med 2011, 108(1):33-36.
4. Pagana KD, Pagana TJ. Mosby's Manual of Diagnostic and Laboratory Tests. Fifth edition. Elsevier Mosby; 2014.
5. Khan SQ, Khan I, Gupta V. CD11b Activity Modulates Pathogenesis of Lupus Nephritis. Front Med 2018, 5:52. https://www.doi.org/10.3389/fmed.2018.00052
6. Fu Y, Du Y, Mohan C. Experimental anti-GBM disease as a tool for studying spontaneous lupus nephritis. Clin Immunol 2007, 124(2):109-118. https://www.doi.org/10.1016/j.clim.2007.05.007
7. Du Y, Fu Y, Mohan C. Experimental anti-GBM nephritis as an analytical tool for studying spontaneous lupus nephritis. Arch Immunol Ther Exp (Warsz) 2008, 56(1):31-40. https://www.doi.org/10.1007/s00005-008-0007-4
8. Peng A, Ye T, Rakheja D, Tu Y, Wang T, Zhou JK, et al. The green tea polyphenol (−)-epigallocatechin-3-gallate ameliorates

experimental immune-mediated glomerulonephritis. Kidney Int 2011, 80(6):601-611. https://www.doi.org/10.1038/ki.2011.121

9. Winther K, Campbell-Tofte J, Vinther Hansen AS. Bioactive ingredients of rose hips (Rosa canina L) with special reference to antioxidative and anti-inflammatory properties: in vitro studies. Bot Targets Ther 2016, 6:11-23. https://www.doi.org/10.2147/BTAT.S91385
10. Longe JL. Gale Encyclopedia of Alternative Medicine. Second edition. Gale; 2005.
11. Fetrow CW, Avila JR. Professional's Handbook of Complementary and Alternative Medicines. Springhouse; 1999.
12. Strålsjö L, Alklint C, Olsson ME, Sjöholm I. Total Folate Content and Retention in Rosehips (Rosa ssp.) after Drying. J Agric Food Chem 2003, 51(15):4291-4295. https://www.doi.org/10.1021/jf034208q
13. Gruenwald J, Uebelhack R, Moré MI. Rosa canina – Rose hip pharmacological ingredients and molecular mechanics counteracting osteoarthritis – A systematic review. Phytomedicine 2019, 60:152958 https://www.doi.org/10.1016/j.phymed.2019.152958
14. Sagdic O, Toker OS, Polat B, Arici M, Yilmaz MT. Bioactive and rheological properties of rose hip marmalade. J Food Sci Technol 2015, 52(10):6465-6474. https://www.doi.org/10.1007/s13197-015-1753-z
15. Larsen E, Kharazmi A, Christensen LP, Christensen SB. An Antiinflammatory Galactolipid from Rose Hip (Rosa canina) that Inhibits Chemotaxis of Human Peripheral Blood Neutrophils in Vitro. J Nat Prod 2003, 66(7):994-995. https://www.doi.org/10.1021/np0300636
16. Jäger AK, Eldeen IMS, van Staden J. COX-1 and -2 activity of rose hip. Phytother Res 2007, 21(12):1251-1252. https://www.doi.org/10.1002/ptr.2236
17. Jäger AK, Petersen KN, Thomasen G, Christensen SB. Isolation of linoleic and α -linolenic acids as COX-1 and -2 inhibitors in rose hip. Phytother Res 2008, 22(7):982-984. https://www.doi.org/10.1002/ptr.2446
18. Fascella G, D'Angiolillo F, Mammano MM, Amenta M, Romeo FV, Rapisarda P, et al. Bioactive compounds and antioxidant activity of four rose hip species from spontaneous Sicilian flora. Food Chem 2019, 289:56-64. https://www.doi.org/10.1016/j.foodchem.2019.02.127
19. Mihaylova D, Vrancheva R, Petkova N, Ognyanov M, Desseva I, Ivanov I, et al. Carotenoids, tocopherols, organic acids, charbohydrate and mineral content in different medicinal plant extracts. Z Für Naturforschung C 2018, 73(11-12):439-448. https://www.doi.org/10.1515/znc-2018-0057
20. Ghendov-Mosanu A, Cristea E, Patras A, Sturza R, Niculaua M. Rose Hips, a Valuable Source of Antioxidants to Improve Gingerbread Characteristics. Molecules 2020, 25(23):5659. https://www.doi.org/10.3390/molecules25235659
21. Grajzer M, Prescha A, Korzonek K, Wojakowska A, Dziadas M, Kulma A, et al. Characteristics of rose hip (Rosa canina L.) cold-pressed oil and its oxidative stability studied by the differential scanning calorimetry method. Food Chem 2015, 188:459-466. https://www.doi.org/10.1016/j.foodchem.2015.05.034
22. Pekacar S, Bulut S, Özüpek B, Orhan DD. Anti-Inflammatory and Analgesic Effects of Rosehip in Inflammatory Musculoskeletal Disorders and Its Active Molecules. Curr Mol Pharmacol 2021, 14(5):731-745. https://www.doi.org/10.2174/1874467214666210804154604
23. Daels-Rakotoarison DA, Gressier B, Trotin F, Brunet C, Luyckx M, Dine T, et al. Effects of Rosa canina fruit extract on neutrophil respiratory burst. Phytother Res 2002, 16(2):157-161. https://www.doi.org/10.1002/ptr.985
24. Guo D, Xu L, Cao X, Guo Y, Ye Y, Chan C-O, et al. Anti-inflammatory activities and mechanisms of action of the petroleum ether fraction of Rosa multiflora Thunb. hips. J Ethnopharmacol 2011, 138(3):717-722. https://www.doi.org/10.1016/j.jep.2011.10.010
25. Willich SN, Rossnagel K, Roll S, Wagner A, Mune O, Erlendson J, et al. Rose hip herbal remedy in patients with rheumatoid arthritis – a randomised controlled trial. Phytomedicine 2010, 17(2):87-93. https://www.doi.org/10.1016/j.phymed.2009.09.003
26. Kirkeskov B, Christensen R, Bügel S, Bliddal H, Danneskiold-Samsøe B, Christensen LP, et al. The effects of rose hip (Rosa canina) on plasma antioxidative activity and C-reactive protein in patients with rheumatoid arthritis and normal controls: A prospective cohort study. Phytomedicine 2011, 18(11):953-958. https://www.doi.org/10.1016/j.phymed.2011.02.008
27. Winther K, Apel K, Thamsborg G. A powder made from seeds and shells of a rose-hip subspecies (Rosa canina) reduces symptoms of knee and hip osteoarthritis: a randomized, double-blind, placebo-controlled clinical trial. Scand J Rheumatol 2005, 34(4):302-308. https://www.doi.org/10.1080/03009740510018624
28. Rossnagel K, Roll S, Willich SN. [The clinical effectiveness of rosehip powder in patients with osteoarthritis. A systematic review]. MMW Fortschr Med 2007, 149(27-28 Suppl):51-56.

29. Zhang GQ, Huang XD, Wang H, Leung AKM, Chan C-L, Fong DWF, et al. Anti-inflammatory and analgesic effects of the ethanol extract of Rosa multiflora Thunb. hips. J Ethnopharmacol 2008, 118(2):290-294. https://www.doi.org/10.1016/j.jep.2008.04.014
30. Lattanzio F, Greco E, Carretta D, Cervellati R, Govoni P, Speroni E. In vivo anti-inflammatory effect of Rosa canina L. extract. J Ethnopharmacol 2011, 137(1):880-885. https://www.doi.org/10.1016/j.jep.2011.07.006
31. Håkansson Å, Stene C, Mihaescu A, Molin G, Ahrné S, Thorlacius H, et al. Rose Hip and Lactobacillus plantarum DSM 9843 Reduce Ischemia/Reperfusion Injury in the Mouse Colon. Dig Dis Sci 2006, 51(11):2094-2101. https://www.doi.org/10.1007/s10620-006-9170-9
32. Kharazmi A. Laboratory and preclinical studies on the anti-inflammatory and anti-oxidant properties of rosehip powder – Identification and characterization of the active component GOPO®. Osteoarthritis Cartilage 2008, 16:S5-S7. https://www.doi.org/10.1016/S1063-4584(08)60003-5
33. Rein E, Kharazmi A, Winther K. A herbal remedy, Hyben Vital (stand. powder of a subspecies of Rosa canina fruits), reduces pain and improves general wellbeing in patients with osteoarthritis—a double-blind, placebo-controlled, randomised trial. Phytomedicine 2004, 11(5):383-391. https://www.doi.org/10.1016/j.phymed.2004.01.001
34. Warholm O, Skaar S, Hedman E, Mølmen HM, Eik L. The Effects of a Standardized Herbal Remedy Made from a Subtype of Rosa canina in Patients with Osteoarthritis: A Double-Blind, Randomized, Placebo-Controlled Clinical Trial. Curr Ther Res 2003, 64(1):21-31. https://doi.org/10.1016/S0011-393X(03)00004-3
35. Schwager J, Richard N, Schoop R, Wolfram S. A Novel Rose Hip Preparation with Enhanced Anti-Inflammatory and Chondroprotective Effects. Mediators Inflamm 2014, 2014:1-10. https://www.doi.org/10.1155/2014/105710
36. Shen Y, Prinyawiwatkul W, Xu Z. Insulin: a review of analytical methods. The Analyst 2019, 144(14):4139-4148. https://www.doi.org/10.1039/C9AN00112C
37. Baynes JW, Dominiczak MH. Medical Biochemistry. Fifth edition. Elsevier; 2019.
38. Gika H, Kaklamanos G, Manesiotis P, Theodoridis G. Chromatography: High-Performance Liquid Chromatography. In: Encyclopedia of Food and Health. Elsevier; 2016:93-99. https://www.doi.org/10.1016/B978-0-12-384947-2.00159-8
39. Yilmaz B, Kadioglu Y, Capoglu I. Determination of Insulin in Humans with Insulin-Dependent Diabetes Mellitus Patients by HPLC with Diode Array Detection. J Chromatogr Sci 2012, 50(7):586-590. https://www.doi.org/10.1093/chromsci/bms042
40. Raja PMV, Barron AR. 4.4: UV-Visible Spectroscopy. Chemistry LibreTexts. [https://chem.libretexts.org/Bookshelves/Analytical_Chemistry/Physical_Methods_in_Chemistry_and_Nano_Science_(Barron)/04%3A_Chemical_Speciation/4.04%3A_UV-Visible_Spectroscopy] Retrieved November 24, 2021.
41. Tabaszewska M, Najgebauer-Lejko D. The content of selected phytochemicals and in vitro antioxidant properties of rose hip (Rosa canina L.) tinctures. NFS J 2020, 21:50-56. https://www.doi.org/10.1016/j.nfs.2020.09.003
42. Benzie IFF, Strain JJ. The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay. Anal Biochem 1996, 239(1):70-76 https://www.doi.org/10.1006/abio.1996.0292
43. Min SY, Yan M, Kim SB, et al. Green Tea Epigallocatechin-3-Gallate Suppresses Autoimmune Arthritis Through Indoleamine-2,3-Dioxygenase Expressing Dendritic Cells and the Nuclear Factor, Erythroid 2-Like 2 Antioxidant Pathway. J Inflamm 2015, 12(1):53. https://www.doi.org/10.1186/s12950-015-0097-9
44. Luckheeram RV, Zhou R, Verma AD, Xia B. CD4+T cells: differentiation and functions. Clin Dev Immunol 2012, 2012:925135. https://www.doi.org/10.1155/2012/925135
45. Tipping PG, James Neale T, Holdsworth SR. T lymphocyte participation in antibody-induced experimental glomerulonephritis. Kidney Int 1985, 27(3):530-537. https://www.doi.org/10.1038/ki.1985.43
46. Klocke J, Kopetschke K, Grießbach AS, Langhans V, Humrich JY, Biesen R, et al. Mapping urinary chemokines in human lupus nephritis: Potentially redundant pathways recruit CD4 $^{+}$ and CD8 $^{+}$ T cells and macrophages: Immunomodulation and immune therapies. Eur J Immunol 2017, 47(1):180-192. https://www.doi.org/10.1002/eji.201646387
47. Gao X, Björk L, Trajkovski V, Uggla M. Evaluation of antioxidant activities of rosehip ethanol extracts in different test systems. J Sci Food Agric 2000, 80(14):2021-2027. https://www.doi.org/10.1002/1097-0010(200011)80:14<2021::AID-JSFA745>3.0.CO;2-2
48. Razungles A, Oszmianski J, Sapis JC. Determination of Carotenoids in Fruits of Rosa sp. (Rosa Canina and Rosa Rugosa) and of Chokeberry (Aronia Melanocarpa). J Food Sci 1989, 54(3):774-775.

https://www.doi.org/10.1111/j.1365-2621.1989.tb04709.x

49. Mihaylova D, Georgieva L, Pavlov A. Antioxidant activity and bioactive compounds of rosa canina l. herbal preparations. Scientific Bulletin Series F Biotechnologies 2015, 19.
50. Taneva, I, Petkova N, Dimov I, Ivanov I, Denev P. Characterization of Rose Hip (Rosa canina L.) Fruits Extracts and Evaluation of Their in vitro Antioxidant Activity. J Pharmacogn Phytochem 2016, 5(2):35-38.
51. Adamczak A, Buchwald W, Zieliński J, Mielcarek S. Flavonoid and Organic Acid Content in Rose Hips (Rosa L., Sect. Caninae Dc. Em. Christ.). Acta Biol Cracoviensia Ser Bot 2012, 54(1). https://www.doi.org/10.2478/v10182-012-0012-0
52. Liu X, Li Y, Wang Y, et al. Gestational vitamin A deficiency reduces the intestinal immune response by decreasing the number of immune cells in rat offspring. Nutrition. 2014;30(3):350-357. https://www.doi.org/10.1016/j.nut.2013.09.008
53. Fan C, Pacier C, M. Martirosyan D. Rose hip (Rosa canina L): A functional food perspective. Funct Foods Health Dis 2014, 4(12):493. https://www.doi.org/10.31989/ffhd.v4i12.159
54. Cohen M. Rosehip - an evidence based herbal medicine for inflammation and arthritis. Aust Fam Physician 2012, 41(7):495-498.
55. Schwager J, Hoeller U, Wolfram S, Richard N. Rose hip and its constituent galactolipids confer cartilage protection by modulating cytokine, and chemokine expression. BMC Complement Altern Med 2011, 11(1):105. https://www.doi.org/10.1186/1472-6882-11-105
56. Wenzig EM, Widowitz U, Kunert O, et al. Phytochemical composition and in vitro pharmacological activity of two rose hip (Rosa canina L.) preparations. Phytomedicine 2008, 15(10):826-835. https://www.doi:10.1016/j.phymed.2008.06.012
57. Winther K, Rein E, Kharazmi A. The anti-inflammatory properties of rose-hip. Inflammopharmacology 1999, 7(1):63-68. https://www.doi.org/10.1007/s10787-999-0026-8
58. Kharazmi A, Winther K. Rose hip inhibits chemotaxis and chemiluminescence of human peripheral blood neutrophils in vitro and reduces certain inflammatory parameters in vivo. Inflammopharmacology 1999, 7(4):377-386. https://www.doi.org/10.1007/s10787-999-0031-y
59. Ye T, Zhen J, Du Y, Zhou JK, Peng A, Vaziri ND, et al. Green Tea Polyphenol (−)-Epigallocatechin-3-Gallate Restores Nrf2 Activity and Ameliorates Crescentic Glomerulonephritis. PLOS ONE 2015, 10(3):e0119543. https://www.doi.org/10.1371/journal.pone.0119543
60. Martirosyan D, Hutcheson J, Sajitharan D, Williams S, Mohan C. The effect of amaranth oil on proteinuria in lupus prone mice. Funct Food Sci 2021, 1(10):39. https://www.doi.org/10.31989/ffs.v1i10.848
61. Chen W, Zhao Y, Dai Z, Zhou Z-S, Zhu P-F, Liu Y-P, et al. Bioassay-guided isolation of anti-inflammatory diterpenoids with highly oxygenated substituents from kidney tea (Clerodendranthus spicatus). J Food Biochem 2020, 44(12). https://www.doi.org/10.1111/jfbc.13511
62. Wang Q, Sun P, Wang R, Zhao X. Therapeutic Effect of Dendrobium candidum on Lupus Nephritis in Mice. Pharmacogn Mag 2017, 13(49):129-135. https://www.doi.org/10.4103/0973-1296.197653
63. Jiang X, Zhao X, Luo H, Zhu K. Therapeutic Effect of Polysaccharide of Large Yellow Croaker Swim Bladder on Lupus Nephritis of Mice. Nutrients 2014, 6(3):1223-1235. https://www.doi.org/10.3390/nu6031223
64. Chen SJ, Aikawa C, Yoshida R, Kawaguchi T, Matsui T. Anti-prediabetic effect of rose hip (Rosa canina) extract in spontaneously diabetic Torii rats: Anti-prediabetic effect of rose hip. J Sci Food Agric 2017, 97(12):3923-3928. https://www.doi.org/10.1002/jsfa.8254
65. Martirosyan D, Kanya H, Nadalet C. Can functional foods reduce the risk of disease? Advancement of functional food definition and steps to create functional food products. Funct Foods Health Dis 2021, 11(5):213. https://www.doi.org/10.31989/ffhd.v11i5.788
66. Sadohara R, Martirosyan D. Functional Food Center's vision on functional food definition and science in comparison to FDA's health claim authorization and Japan's Foods for Specified Health Uses. Funct Foods Health Dis 2020, 10(11):465. https://www.doi.org/10.31989/ffhd.v10i11.753
67. Martirosyan D, Liufu J. FFC's Advancement of the Establishment of Functional Food Science. Funct Foods Health Dis 2020, 10(8). https://www.doi.org/10.31989/ffhd.v10i8.729