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The effect of amaranth oil on proteinuria in lupus prone mice

Danik Martirosyan*[1,2], Jack Hutcheson[1], Deena Sajitharan[1], Samantha Williams[2], Chandra Mohan[1]

[1]Department of Internal Medicine, The University of Texas Southwestern Medical Center, Dallas, TX, 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

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ABSTRACT

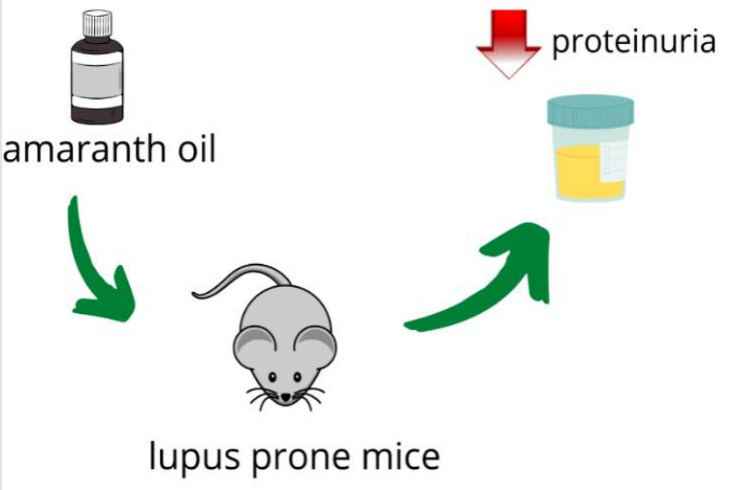
Background: Kidney disease is a leading cause of death in the United States and the world. Proteinuria signifies kidney damage and can exacerbate kidney disease. It has become an important indicator of kidney disease; reducing proteinuria results in renoprotective effects and slows renal disease. Existing treatments do not work for every patient.

Aim of Study: Amaranth is a tropical plant that was regularly consumed in the ancient Central American diet. It has a plethora of health effects and is a strong functional food candidate. This study examines whether a regular oral dose of amaranth oil decreased proteinuria concentration in murine subjects.

Methods: Mice in the experimental group (n = 3) were given 4µl of amaranth oil per gram of mouse weight for 5 days a week over 84 days. Control mice (n = 2) were sham treated on the same schedule with no oil. Urine protein concentration was determined by Bradford assay, measuring absorbance at 595nm, then comparing with a BSA standard curve.

Results: The experimental group showed decreased proteinuria levels throughout the entire 84 days of study

Conclusion: Results show amaranth oil may help decrease proteinuria levels in lupus prone murine subjects. Given the small sample size, the data are preliminary. More research is needed to validate the results and determine the optimal dose and treatment schedule.



Key words: proteinuria, amaranth oil, renoprotective, kidney disease, renal disease, lupus, functional food

INTRODUCTION

Kidney disease is the 8th leading cause of death in the United States and 10th in the world [1-2]. Proteinuria is defined as the presence of proteins in the urine [3]. It signifies kidney damage and can exacerbate kidney disease [4-6]. In addition, proteinuria has been shown to increase the risk of renal impairment, hypertension, and cardiovascular disease [5, 7-10]. It has become an important indicator of kidney disease, may help diagnose preeclampsia, and is common in kidney transplant recipients [5, 8-9, 11-14]. In kidney transplant patients, proteinuria is associated with decreased kidney survival, significantly greater risk of graft loss, and increased patient death [14-17]. Reducing proteinuria results in renoprotective effects and slows renal disease [5, 18]. Conditions that can lead to proteinuria include immune disorders, such as systemic lupus erythematosus (SLE), as well as preeclampsia, diabetes, and increased oxidative stress [19-20].

Current treatments include various medications and dietary changes [7, 18]. The general treatment of choice is to inhibit the renin-angiotensin-aldosterone system (RAAS) via an angiotensin-converting enzyme inhibitor or angiotensin II receptor blocker, but it can be unsafe or ineffective for certain patients [9, 21-25]. Another treatment is anti-vascular endothelial growth factor (VEGF) therapy, but it may be related to kidney injury and chronic use can exacerbate interstitial fibrosis [9, 26]. Specific treatments include angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers, restricting dietary protein and sodium, lowering blood pressure, renin inhibitors, aldosterone antagonists, non-dihydropyridine calcium channel blockers, and pentoxifylline [14, 25, 27-34]. In kidney transplant

patients, ACE inhibitors, angiotensin receptor blockers, and restricting dietary protein have also been shown to decrease proteinuria [14]. While further study might be warranted for these treatments, it would be ideal to have alternatives. In other studies, hydroxychloroquine and curcumin were successful in decreasing proteinuria [35-36].

The SLE autoimmunity and inflammatory processes are related to changes in the lipid profile, specifically alteration in lipid metabolism and increased oxidative stress [37-38]. Notably, in patients with lupus, oleic acid is decreased and linoleic acid is associated with higher disease activity [39-40]. In addition to anticancer and anti-inflammatory qualities, conjugated linoleic acid has previously been shown to decrease SLE related oxidative stress via activating detoxifying enzymes and improving antioxidant status [41]. Additionally, past studies found that SLE patient serum had lower α-tocopherol concentration, as well as decreased plasma antioxidant status due to lower plasma superoxide dismutase, and glutathione peroxidase activity [38, 42-43].

Amaranth is an ancient tropical plant that was regularly consumed by the Aztecs, Inca, and Maya [44-45]. The food value of 100g of amaranth oil is noted in Table 1. Notably, compared to cereal alternatives, the grain has more proteins, lipids, and minerals [46]. It is rich in antioxidants and phytochemicals, and has previously been shown to have antihypertensive, antidiabetic, and anticholesteremic effects [47-52]. These properties have been attributed to amaranth having ample monosaturated fatty acids, polyunsaturated fatty acids, squalene, linoleic acids, and essential amino acids [47-48, 53]. Major fatty acids in amaranth oil include palmitic acid (19.1−23.4%), oleic acid (18.7−38.9%), and linoleic acid (36.7−55.9%) [47, 54]. It was also reported to have 2.4-8% squalene, more than other vegetable oils [54]. The chemical structure of squalene is shown in Figure 1. Furthermore, amaranth contains tocopherols and tocotrienols [47, 52, 55-57], which are isoforms of vitamin E and exhibit antioxidant activity [58]. Particularly, they can protect against lipid peroxidation on cell membranes, preventing oxidative damage [59].

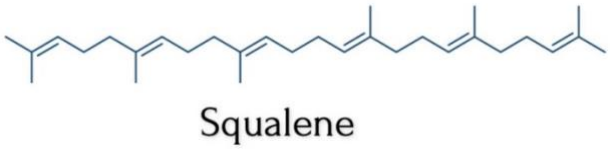


Figure 1. The chemical structure of squalene. Amaranth oil is particularly rich in squalene (2.4-8%).

In addition, the omega-6 to omega-3 ratio for amaranth ranges from 54.9 to 68.63, considerably higher than that of many cereals [60]. Martirosyan and co-authors found that changes in the fatty acid composition of erythrocyte membranes due to amaranth oil were only evident with 18ml of oil daily; these changes were partly due to squalene [47]. Lee et al found that while omega-3 fatty acid supplementation did not seem to affect proteinuria in diabetes mellitus patients, it did result in significantly higher oleic acid and omega-6 fatty acid erythrocyte membrane contents [61].

Finally, amaranth contains bioactive peptides [46, 62-64]. Previous researchers found amaranth derived peptides and tetrapeptides with ACE-inhibitory activity

[65-67]. Others found a variety of potential effects including antihypertensive, antioxidative, and antithrombotic [65, 68]. Moreover, a number of researchers investigated emulsions and foods made with amaranth and found they still show antihypertensive effects [46, 63, 69-72]. Overall, amaranth has considerable potential as a functional food and warrants further research. This study examines whether a regular oral dose of amaranth oil decreased proteinuria concentration in murine subjects.

METHODS and MATERIALS

Mice: B6.Sle1.Sle2.Sle3 mice (n = 5) were bred and housed at University of Texas Southwestern Medical Center. Mice were aged to 7-9 months of age prior to the start of the study and all experiments were performed on male mice. All studies were conducted with the prior approval of the University of Texas Southwestern Medical Center Institutional Animal Care and Use Committee.

This study did have a 1µl experimental group (data not presented), which showed results in line with the presented data. It is also important to note that the mice in this study were lupus prone, so it is possible that results may differ in non-lupus prone subjects.

Amaranth Oil Treatment: Amaranth oil extracted from *Amaranthus hybridus* L. was obtained from Russian Oliva, Voronezh Russian Federation. Mice in the experimental group (n = 3) received 4µl of amaranth oil per gram of mouse weight by oral gavage 5 days per week over the course of 84 days. Control mice (n = 2) were sham treated on the same schedule with an oral gavage needle containing no oil.

Table 1. Food Value of 100g Amaranth Oil (modified from [47])

Food Value	Amaranth Oil
Squalene (g)	5.9
Phospholipids (g)	8
Phytosterols (g)	2
Sum of tocopherols (vitamin E), in mg	300

Proteinuria: Urine was collected biweekly from mice placed in metabolic cages for 24 hours. Mice fasted for the duration of the urine collection to prevent chow debris from contaminating the urine samples with protein. Urine volume was recorded at the time of collection and protein concentration was determined by Bradford assay (Thermo Fisher Scientific, Rockford, IL). Five microliters of urine was added in duplicate to a 96-well microplate and 200 microliters of Bradford reagent was mixed on top of each sample. The absorbance was read at 595 nm and the protein concentration was determined by comparison with a BSA standard curve. Each protein concentration (µg/ml) was then multiplied by the recorded volume to determine the final concentration (µg/24 hours).

RESULTS AND DISCUSSION

The experimental group showed decreased proteinuria levels throughout the entire 84 days of study, shown in Figure 2. As depicted in Table 2, proteinuria levels were on average 226.9µg lower per day; the smallest difference observed was 93.2µg on the 28th day, while the largest difference was 427.16µg on the 42nd day.

Table 2. Urine protein levels (µg/day) for experimental and control groups over the 84 days of study.

	D0	D14	D28	D42	D56	D84	Average
No Oil	847.8203	911.6238	1038.337	1704.216	1048.22	862.0306	1068.7078
4µl/g	652.9582	794.9571	945.1064	1277.058	719.4237	661.2754	841.79636
Difference	194.8621	116.6667	93.23017	427.158	328.7964	200.7552	226.91143

The proteinuria difference started at 194.86µg, but lessened to 116.67µg on day 14, and further reduced to 93.23µg on day 28. On day 42, the difference increased to 427.16µg, then lessened to 328.8µg on day 56, before decreasing to 200.76µg on day 84.

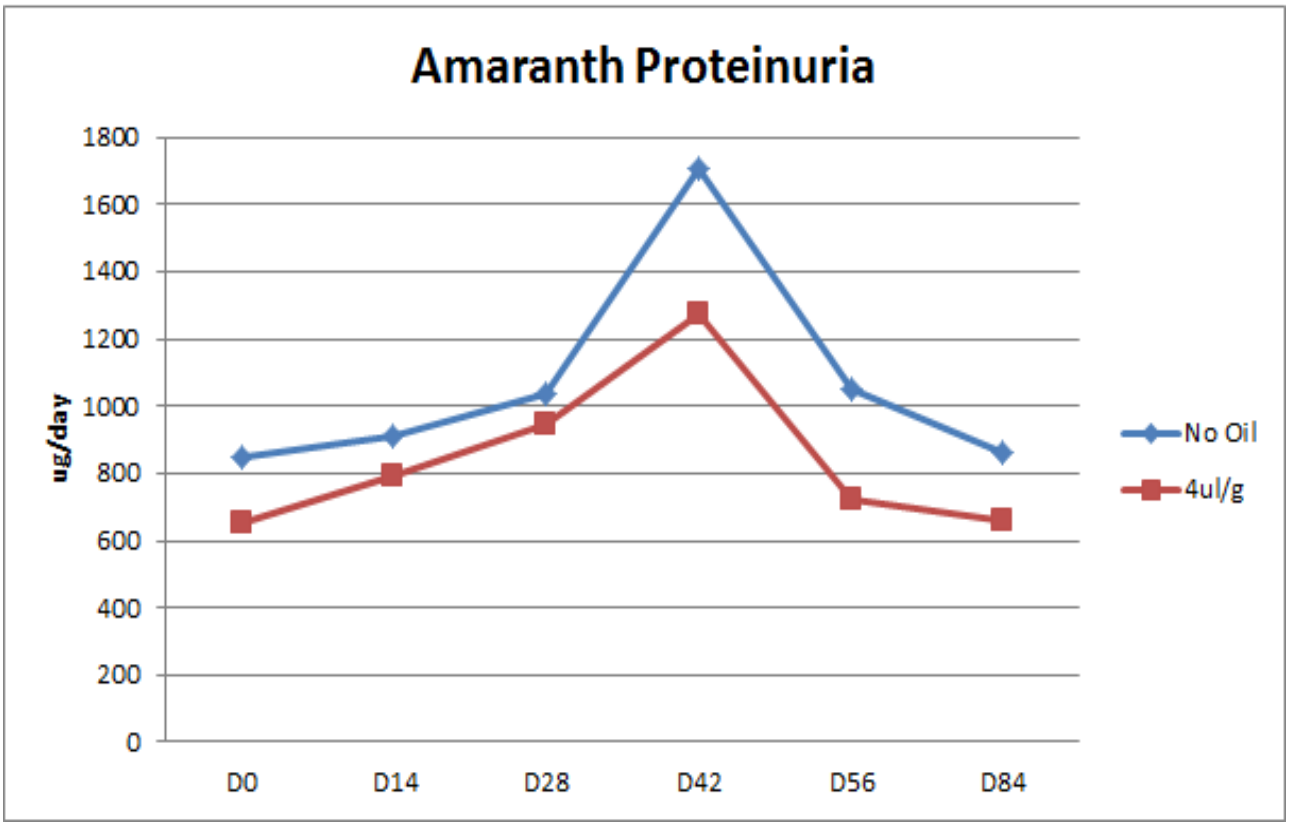


Figure 2. Proteinuria levels for experimental and control groups over the 84 days of study.

The experimental group had lower proteinuria levels than that of the control throughout the entire length of the study, with the biggest difference observed on day 42. From day 0 to day 28, the difference gradually reduced until increasing on day 42. The difference lessened from day 42 to day 56 and continued to do so between day 56 to day 84.

The study originally had two dosage groups, 4µl and 1µl, with three mice each, as well as a control group with two mice. Two mice in the 1µl group passed away, resulting in n = 1 for that group; the data are hence not reported, but are in line with the presented data. The presented data thus include n = 3 for the 4µl experimental group and n = 2 for the control group.

Previously, it has been shown that curcumin decreased proteinuria levels [35], but such data with amaranth oil has not been presented. Results show that compared to the control, the group receiving 4µl amaranth oil per gram of mouse weight had decreased proteinuria levels for the entire 84 days of study. Given amaranth's various health benefits, the results are not too surprising. However, the exact cause of these effects is not certain.

As mentioned earlier, amaranth possesses phytochemicals and antioxidants [47, 49, 52]. Numerous studies have illustrated the renoprotective effects of antioxidative and phytochemical agents [73-77]. Particularly, amaranth oil has been shown to reduce oxidative stress and lend an antioxidant protective effect in rat kidney, plasma, and pancreas [73, 78]. Similarly, it has been observed that grain amaranth's antioxidant activity protects blood, kidney, and liver tissues in mice with diabetes mellitus [50, 73]. Kosozi et al suggested that this is because grain amaranth aids calcium homeostasis in blood, kidney, and liver leading to antidiabetic effects [50].

Additionally, proteinuria can be decreased with ACE and renin inhibitors [14, 25, 27-28, 34]. Past studies demonstrated that the bioactive peptides in amaranth proteins display ACE inhibiting effects *in vitro* and *in vivo* [46, 62-63]. Quiroga et al found that amaranth peptides also inhibit renin of the RAAS in dose-response fashion via competitive inhibition; inhibition efficacy was determined to be directly related to peptide hydrophobicity [46, 64]. Though ACE and RAAS inhibitory activity has been observed, amaranth's effects on proteinuria were not previously explored.

Proteinuria is an important indicator of kidney damage and disease, as well as hypertension and cardiovascular disease. It is also associated with several negative outcomes in kidney transplant patients. Reducing proteinuria helps protect the kidney and is effective in slowing renal disease. However, current treatments can be unsafe or ineffective, so alternatives would be ideal.

Amaranth oil is a strong functional food candidate. In addition to numerous health benefits, it exhibits antioxidant protective effects and contains several bioactive compounds, including squalene. Several investigations showed amaranth oil's health properties and functionality. Particularly, it was shown that it can decrease the amount of total cholesterol, low density cholesterol (LDL), and triglycerides significantly [47]. In addition, it was shown that amaranth oil has hepatoprotective effects, which were confirmed by biological and morphological examination [79]. In the current investigation, we have shown that amaranth oil may help decrease proteinuria levels in lupus prone murine subjects. However, according to the Functional Food Center's/Dr. Martirosyan's definition of functional foods, we should determine exactly which bioactive compounds and what non-toxic dosages provide a

clinically proven and documented health benefit, utilizing specific biomarkers [80-82]. At this point, our data are preliminary, based on a very small sample size, and also clinically were not approved for lupus patients, so we are not able to claim amaranth/amaranth oil functionality in the case of lupus disease. In the future, similar investigations using a larger sample size will be needed, and if it is confirmed that amaranth oil decreases proteinuria levels in lupus prone murine subjects, then several clinical studies should be done to confirm amaranth oil effectiveness for proteinuria.

In conclusion, our results show that amaranth oil may help decrease proteinuria levels in lupus prone murine subjects. However, the data are preliminary. Future studies should determine if the effects are observed in other subjects and in a larger sample size. The optimal dosage and treatment schedule should also be investigated.

Abbreviations: RAAS: renin-angiotensin-aldosterone system, VEGF: anti-vascular endothelial growth factor, ACE: angiotensin-converting enzyme, SLE: systemic lupus erythematosus, LDL: low density cholesterol

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

Authors' contribution: The original idea was conceived by DM and was discussed with CM. The main focus and ideas of the experiments finally agreed with JH and DS. The experiments were conducted and analyzed by DS, JH and DM. Experimental data was analyzed by DS and JH, and discussed with DM and CM. The main text of the paper including methods were written by SW, JH, and DM. The manuscript was revised, edited, and formatted by DM and SW.

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