Chapter

Teucrium ramosissimum Derived-Natural Products and Its Potent Effect in Alleviating the Pathological Kidney Damage in LPS-Induced Mice

Fatma Guesmi and Ahmed Landoulsi

Abstract

Teucrium esssential oil mediates an extensive spectrum of biological effects, including renal diseases. The aim of this research was to explore the ethnobotanical feature, biochemical composition and antiinflammatory potential of *T. ramosissimum* alone or prior the use of LPS-induced kidney damage. The essential oils were subjected to Gas chromatography-mass spectrometry (GC/MS) apparatus to detect biomolecules in *T. ramosissimum*. *In vivo* renal dysfunction induced by LPS was investigated using mouse model. Our data showed that oral treatment of animals with LPS highly increased level of serum biomarkers and induces renal dysfuntion, whereas, pre-treatment with *T. ramosissimum* mediated markedly histopatological changes of kidney architecture and ameliorates renal function. Dense cover of secretory structures in *teucrium* leaves may protect this specie. Overall, this study showed phytocompounds richness and interesting biological activities of Tunisian *Teucrium ramosissimum*. Essential oil of this specie *T. ramossimum* given prior to LPS exposure protected mice from renal inflammation.

Keywords: *Teucrium ramosissimum*, essential oil, hairs, LPS, renal dysfunction

1. Introduction

The genus *Teucrium L.* (Lamiaceae) is a genus growing in mild climate zones, particularly in the Mediterranean Basin and Central Asia [1]. Limited number of in-depth scientific researches have been done so far on the phytochemistry and bioactivities [2] of *Teucrium* genus that is represented by herbs or shrubs, with tubular or campanulate calyx, 2-lipped or actinomorphic, 5-toothed, the teeth equal or the upper larger; corolla with one 5-lobed lip; tube without a ring of hairs inside, often included in the calyx; and nutlets smooth or reticulate [1].

Teucrium species have been used in phytopharmacology, helping to treat many diseases, including tuberculosis, gastrointestinal disorders, inflammations, rheumatism, and diabetes [3]. The widespread applications of *Teucrium* genus in the ethnomedicine

of several countries [1] may be due to its richness in biocompounds used in *in vivo* and *in vitro* biological effects. Moreover, traditional health care systems based on plants and plant-derived products are highly popular and employed therapeutically [2] in Tunisia. The plant essence that contains several secondary metabolites is synthetized in all parts (leaves, flowers, stem, seeds, buds). The use of essential oils in industries are markedly increased, including the beverage, food, aromatherapy, cosmetics and personal care [4]. Moreover, the recent trend in the field of inflammation research is to search for alternative therapeutic agents from natural sources that are devoid of the adverse effects characteristic for conventional steroids or nonsteroidal anti-inflammatory drugs (NSAIDs). In this context, phytochemical studies and biochemical investigations on the mode of action of traditional complementary remedies are of utmost importance [2]. Acute kidney damage, a great public health problem, has been grown in the world. It's a critical care syndrome and an abrupt loss in renal function [5], resulting in acute reduction of renal activity and up to 22% mortality of hospitalized patients. Acute kidney injury is estimated to occur in about 20–200 per million population in the community, 7–18% of patients in hospital, and approximately 50% of patients admitted to the intensive care unit (ICU) [6]. Lipopolysaccharide (*Escherichia coli* 055:B5) is one of the most important causes of sepsis and is involved in the pathogenesis of sepsis-associated acute kidney injury (SA-AKI), which may lead to "cytokine storm," intensified oxidative stress, low blood pressure, renal hypoperfusion, and finally a gradual decline in renal function [7].

T. ramosissimum belongs to the Lamiaceae family, of the genus *Teucrium* is known as "Hchichet Belgacem" or "Hchichet Ben Salem" in the region of Gafsa in the southwest of Tunisia [8]. The specie is present in the South of Tunisia in particular in Djebel Orbata (Zannouch-Gafsa, Tunisia) and Bou Hedma Mount (Sidi Bouzid-Tunisia). It exists as a small sub-shrub, bushy, of silver gray, 8–15 cm tall. The stems are slender, erect and small. While, the leaves are white, with rounded limb with 7 deep crenellations. The inflorescence is pauciflor; the white calyx is 4 mm long, with long acute and sub-natural teeth [8]. Three sesquiterpenoids (teucmosin, 4α-hydroxy-homalomenol C, 1β,4β,7α-trihydroxy-8,9-eudesmene), five sesquiterpenoids (oplopanone, homalomenol C, oxo-*T*-cadinol, 1β,4β,6β-trihydroxyeudesmane, 1β,4β,7α-trihydroxyeudesmane) and two trinorsesquiterpenoids (4β-hydroxy-11,12,13-trinor-5-eudesmen-1,7-dione and 1β,4β-dihydroxy-11,12,13-trinor-8,9-eudesmen-7-one) were isolated from the ethanolic extracts of the aerial parts of *Teucrium ramosissimum* [9]. *Teucrium ramosissimum* is particularly present in the higher mounts of southern Tunisia. The ethnopharmacological uses of this specie in Tunisia are for treatment of inflammation. In fact, many people apply the powder of this species on the external inflamed area to reduce swelling and pain.

The present study provides a new insight into the organ architecture of *Teucrium ramosissimum* Desf. Biochemical compounds of *T. ramosissimum* leaves were detected using GC/MS apparatus. Histological analysis and enzyme levels showed that *T. ramosissimum* decreased LPS-mediated acute kidney injury by inhibiting tissues inflammation and reducing kidney tissue damage.

2. Materials and methods

2.1 Chemicals

T. ramosissimum essential oil solutions (100 mg/ml) were diluted in dimethyl sulfoxide for *in vivo* analysis. 5-Fluorouracil (5-FU) and LPS (*Escherichia coli* 055:B5) was obtained from Sigma-Aldrich Chemicals Co. (St. Louis, MO, USA).

2.2 Plant materials

Leaves of *T. ramosissimum* were collected from the mount of Orbata (Gafsa, Tunisia) during the Springer (2018). Essential oil was extracted by Clevenger apparatus. Characterization of phytochemicals by GC-MS analysis indicated the presence of mono- and sesquiterpenic compounds.

2.3 Analyses of oily fractions of *T. ramosissimum* with GC/MS

Oily fractions (diluted in 10% hexane) were analysed using GC/MS on a model 6890 gas chromatograph with an autosampler coupled with an Agilent 5973 Mass Selective *Detector* (Agilent Technologies, Palo Alto, CA, USA) with an electron impact ionization of 70 eV. A Phenomenex capillary column, ZB-5MSi (30 m × 250 μm i.d., 0.5 μm film thickness) (Agilent Technologies, Hewlett-Packard, CA, USA) at a temperature rising from 40 to 280 °C (5 °C/min). The carrier gas at a purity of 99.999% used for GC/MS analyses was helium at a flow rate of 0.7 ml/min, a scan time of 1 s and mass range *m/z* 50–550. The terpenic compounds were identified by matching their retention indices with those of the Wiley 09 NIST 2011 mass spectral library of the apparatus.

2.4 Protective effects of *T. ramosissimum* against LPS-induced renal inflammation

2.4.1 Experimental design

Both sexes of Swiss albino mice (48, 25 g weight) were divided into 8 groups (n=6) and maintained in plastic cages (polypropylene). Mice, provided from Pasteur Animal Laboratory (Tunisia, Ethic# LNSP/Pro 152012), were housed under animal conditions (25 ± 5°C; 45–55 % relative humidity; 12 h light/dark cycles with free access to water and food.

Group 1: Normal control, orally treated with saline;
Group 2: negative control, orally treated with 10 μg/ml LPS;
Group 3: orally treated with 20 μg/kg *T. ramosissimum* essential oil diluted in Tween 80 (2%);
Group 4: orally treated with 50 μg/kg *T. ramosissimum* essential oil diluted in Tween 80 (2%);
Group 5: comparator control, orally treated with 20 mg/ kg/day 5-FU;
Group 6: orally treated with the mixture of LPS and *Teucrium* essential oil (10 μg/ml and 20 μg/kg, respectively);
Group 7: orally treated with the mixture of LPS and *Teucrium* essential oil (10 μg/ml and 50 μg/kg, respectively);
Group 8: orally treated with the mixture of LPS and 5-FU (10 μg/ml and 20 mg/kg, respectively).

Animals received drugs for one week. In the groups 6, 7 and 8, mice were treated with *Teucrium* essential oil or 5-FU 1 hour before LPS administration.

At the 8th day, mice were sacrificed and blood samples were collected by glass capillary tubes for plasma biomarker analysis. Kidney tissues were collected and processed for microscopic analysis.

2.5 Statistical analyses

Statistical analyses of *in vivo* study were performed using GraphPad Prism 4.00 to compare different groups with each other we used a two-way analysis of variance (ANOVA), followed by Tukey's multiple test. A value of $P < 0.05$ was considered statistically significant.

3. Results and discussion

3.1 Ethnobotanical and phytochemical analysis of *T. ramosissimum*

T. ramosissimum macromorphological features (nutlets, leaves, stems, flowers) are shown in **Figure 1**. The major phytocompounds were β-phellandrene, α-cadinol, T-Muurolol, α-bisabolol, camphor, endo-borneol, and epi-bicyclosesquiphellandrene (**Figure 2**).

3.2 Effects of *T. ramosissimum* on histological changes of cecum and serum biomarkers of the kidneys

LPS mediated a significantly ($P<0.05$) increase in the levels of plasma urea, creatinine and uric acid (**Figure 3A**). The pretreatment of mice with *T. ramosissimum* notably reduced the levels of plasma biomarkers in serum. Macroscopic features of kidney taken from treated and untreated groups are shown in **Figure 3Bi**. As depicted in **Figure 3Bii**, normal glomerular histoarchitectural and numerous tubules was seen in the kidney and a significant increase in the tubular injury scores after LPS treatment, otherwise, we noted fibrotic lesions, leukocyte infiltration indicative of the inflammation within different areas in the glomeruli, and renal tubules degeneration associated to tubular epithelium desquamation, while no observed damage was detected in mice from normal control group. After one week of treatment of mice with LPS, pronounced tubular necrosis and kidney fibrotic scaring was observed in kidney, which was significantly reversed by *T. ramosissimum* treatment that improves significantly the renal functions when it was given prior to LPS administration (**Figure 3Biii**).

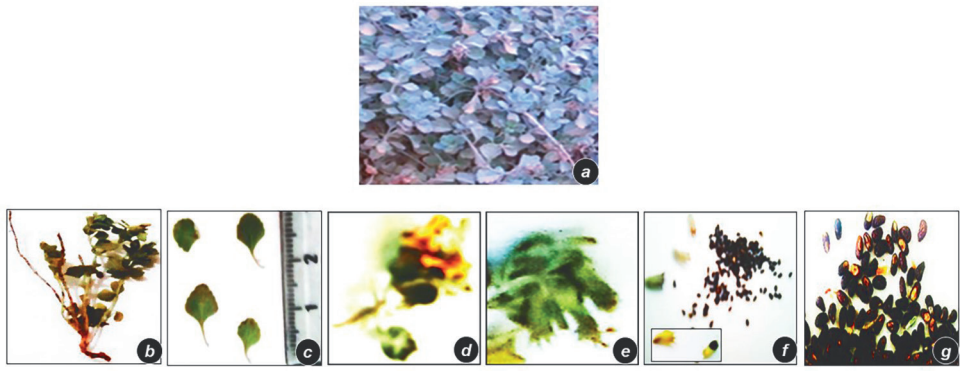

Figure 1.
T. ramosissimum *plant. a: habitus; a: leaves; b,c: leaves; d,e: seeds.*

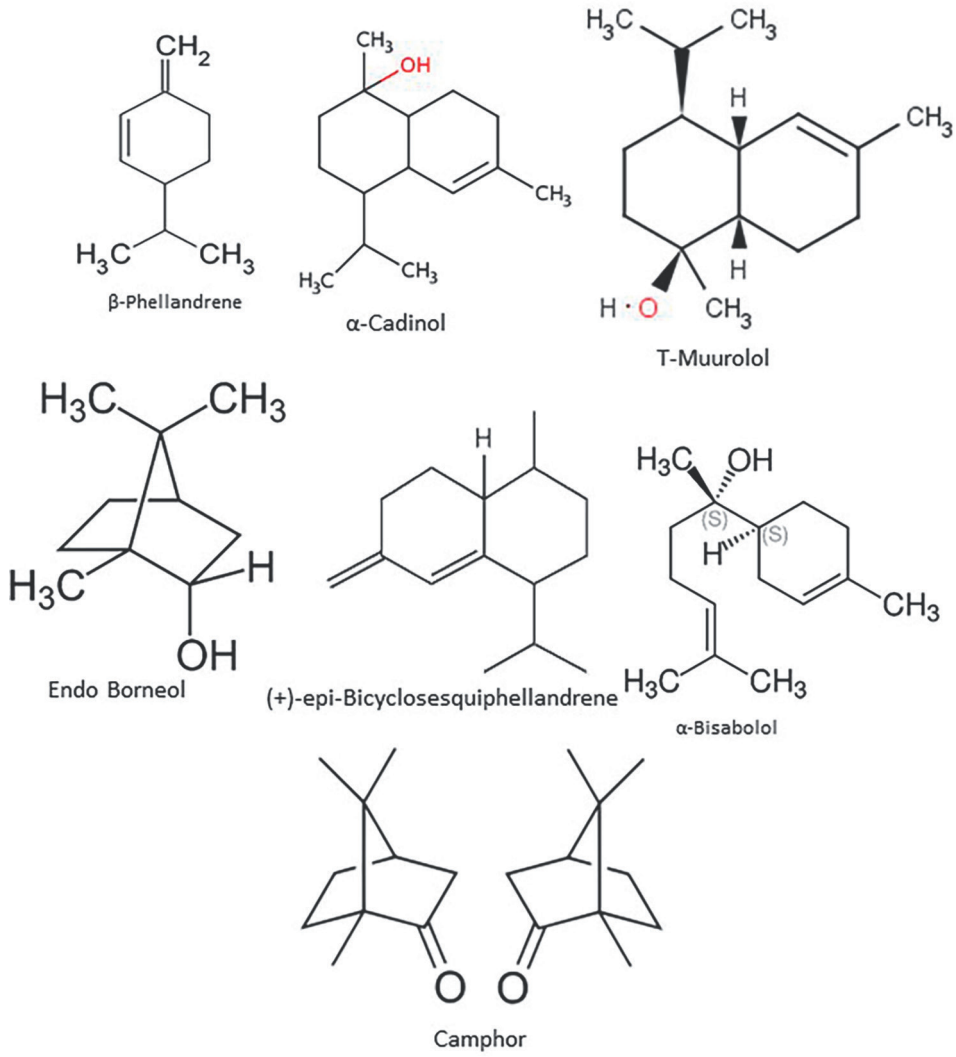

Figure 2.
Phytocompounds of T. ramosissimum *essential oil isolated using GC/MS analysis.*

Free radicals- induced lipid peroxidation to be one of the major causes of cell membrane damage resulting in a series of pathological situations by causing acute and chronic renal injuries [10]. In fact, LPS is one of the most important causes of sepsis and is involved in the pathogenesis of SA-AKI, which may lead to "cytokine storm," intensified oxidative stress, low blood pressure, renal hypoperfusion, and finally a gradual decline in renal function [7]. In this report, photomicrograph overview revealed the potent protective effect of *T. ramosissimum* that could effectively attenuate the pathological cecum and renal alteration in LPS-induced colorectal inflammation and acute kidney injury in mice model by reducing inflammation. These imply that *T.ramosissimum* pretreatment may attenuates the pathological features of LPS-induced inflammation revealed by Hematoxylin & Eosin (H&E) staining.

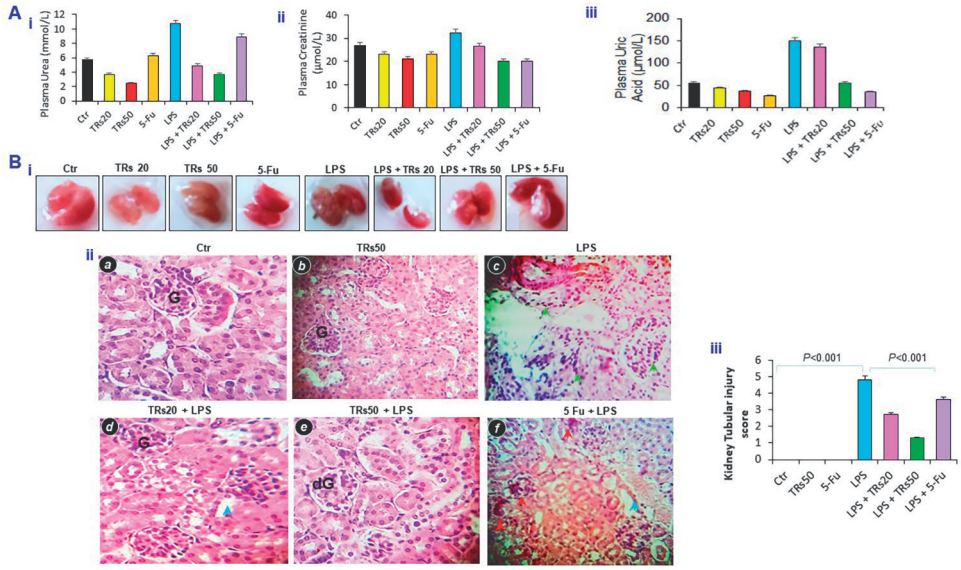

Figure 3.
A. Creatinine (i), urea (ii) and UA (iii) levels in plasma. B. Macroscopical view of mice kidney (i), histopathological analysis (ii) of kidney sections (scale bar = 250 µm) observed by H&E staining and Kidney tubular injury score (iii) of treated groups. Green arrows- inflammatory cell infiltration; red arrows: hemorage; blue arrows: edema of the intertubular spaces; TRs: Teucrium ramosissimum*; G: glomerulus; dG: degenerated glomerulus. (H&E staining, Magnification: a–e ×40; f ×10). Data represent mean ± SD, n=6; ** P<0.01 vs. LPS.*

The current results further support previous findings on the effect of LPS to mediate tubule and glomerulus degeneration.

T. ramosissimum given prior to LPS treatment induced decrease in serum biomarkers (urea, uric acid, creatinine). In this report, plasma creatinine and urea increased significantly in LPS-treated group, and this indicate diminished ability of the kidneys to filter these waste products from the blood and excrete them in the urine [11]. Additionally, this work demonstrated that LPS increased uric acid that mediated arteriolopathy and interstitial inflammation suggest mechanisms that would exacerbate or potentiate progressive renal functional decline after injury. This process involves accumulation of free radicals. Moreover, in the kidney, LPS binds to TLR4 proteins and mediates the proinflammatory cytokines release, and more precisely IL-1β and TNF-α [7] and induces the transcriptional factor, NF-kB, activation that regulates a variety of inflammatory gene expression [12]. Effectively, any compound able to modulate inflammation or inflammation-related processes can be thought of as a renal protective agent and/or a potential treatment tool for controlling renal damage [13].

T. ramosissimum is traditionally used for the treatment of many diseases (inflammation, gastric ulcer, cancer). Its extracts markedly enhance cell proliferation either with or without mitogen (lipopolysaccharide [LPS] or lectin) stimulation and contain potent components such as flavonoids that may be potentially useful for modulating immune cell functions in physiological and pathological conditions. Moreover, *Teucrium* extract exert different protective effects against ethanol-induced ulcerogenesis [14]. Likewise, this species acts as chemopreventive and chemosensitizing agent against two uterine sarcoma cell lines, MES-SA and P-gp-overexpressing MES-SA/Dx5 cells by a slight modulation of the cell cycle and its regulators, but also through a significant induction of apoptosis [15].

4. Conclusions

This report affirms that phytpharmacological effects of *Teucrium* essential oil extracted at the flowering stage may be related to its derived products identified by GC/MS apparatus. *T. ramosissimum* can restore LPS-induced renal damage by inhibiting inflammation. The increase of serum biomarkers, together with glomerular and tubular alterations clearly indicate renal dysfunction.

Acknowledgements

The authors extend their appreciation to The Ministry of Higher Education and Scientific Research of Tunisia.

Conflict of interest

The authors declare no conflict of interest.

Author details

Fatma Guesmi* and Ahmed Landoulsi
Faculty of Sciences of Bizerte, Laboratory of Risks Related to Environmental Stresses: Fight and Prevention, University of Carthage, Carthage, Tunisia

*Address all correspondence to: guesmif6@gmail.com

IntechOpen

© 2022 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. CC BY

References

[1] Maccioni A, Falconieri D, Sanna C, Porcedda S, Piras A, Maxia A. Characterization of essential oils from different taxa belonging to the genus *teucrium* in Sardinia Island, Italy. Plants. 2021;**10**:1359. DOI: 10.3390/plants10071359

[2] Napagoda M, Gerstmeier J, Butschek H, De Soyza S, Pace S, Lorenz S, et al. The anti-inflammatory and antimicrobial potential of selected ethnomedicinal plants from Sri Lanka. Molecules. 2020;**25**:1894. DOI: 10.3390/molecules25081894

[3] Jarić S, Mitrović M, Pavlović P. Ethnobotanical features of *teucrium* species. In: Stanković M, editor. Teucrium Species: Biology and Applications. Cham: Springer; 2020. DOI: 10.1007/978-3-030-52159-2_5

[4] Irshad M, Ali Subhani M, Ali S, Hussain A. Biological Importance of Essential Oils. Rijeka: Intechopen; 2019. DOI: 10.5772/intechopen.87198

[5] Fortrie G, de Geus HRH, Betjes MGH. The aftermath of acute kidney injury: A narrative review of long-term mortality and renal function. Critical Care. 2019;**23**:24. DOI: 10.1186/s13054-019-2314-z

[6] Chawla LS, Bellomo R, Bihorac A, Goldstein SL, Siew ED, Bagshaw SM, et al. Acute kidney disease and renal recovery: Consensus report of the acute disease quality initiative (ADQI) 16 workgroup. Nature Reviews Nephrology. 2017;**13**:241-257

[7] Chen Y, Jin S, Teng X, Hu Z, Zhang Z, Qiu X, et al. Hydrogen sulfide attenuates LPS-induced acute kidney injury by inhibiting inflammation and oxidative stress. Oxidative Medicine and Cellular Longevity. 2018;**2018**:10. DOI: 10.1155/2018/6717212

[8] Ben Sghaier M, Louhichi T, Ammari Y. Ethnobotanical and phytopharmacological notes on *teucrium ramosissimum* L. Research Review Biosciences. 2017;**12**(3):132

[9] Henchiri H, Bodo B, Deville A, Dubost L, Zourgui L, Raies A, et al. Sesquiterpenoids from *teucrium ramosissimum*. Phytochemistry. 2009;**70**(11-12):135-1441

[10] Farzaei MH, Zangeneh MM, Goodarzi N, Zangeneh A. Stereological assessment of nephroprotective effects of trachyspermum ammi essential oil against carbon tetrachloride-induced nephrotoxicity in mice. International Journal of Morphology. 2018;**36**(2):750-757

[11] El-Demerdash F, Dewer Y, ElMazoudy RH, Attia AA. Kidney antioxidant status, biochemical parameters and histopathological changes induced by methomyl in CD-1 mice. Experimental Toxicology and Pathology. 2013;**65**:897-901

[12] Chunzhi G, Zunfeng L, Chengwei Q, Xiangmei B, Jingui Y. Hyperin protects against LPS-induced acute kidney injury by inhibiting TLR4 and NLRP3 signaling pathways. Oncotarget. 2016;**7**(50):82602-82608

[13] Avila-Carrasco L, García-Mayorga EA, Díaz-Avila DL, Garza-Veloz I, Martinez-Fierro ML, González-Mateo GT. Potential therapeutic effects of natural plant compounds in kidney disease. Molecules. 2021;**26**:6096. DOI: 10.3390/molecules26206096

[14] Ben Sghaier M, Harizi H, Louhichi T, Krifa M, Ghedira K, Chekir-Ghedira L. Anti-inflammatory and antiulcerogenic activities of leaf extracts and sesquiterpene from *teucrium ramosissimum* (Lamiaceae). Immunopharmacology and Immunotoxicology. 2011;**33**(4):656-662. DOI: 10.3109/08923973.2011.558903

[15] Lahmar A, Mathey A, Aires V, Elgueder D, Vejux A, Khlifi R, et al. Essential oils, *pituranthos chloranthus* and *teucrium ramosissimum*, chemosensitize resistant human uterine sarcoma MES-SA/Dx5 cells to doxorubicin by inducing apoptosis and targeting P-glycoprotein. Nutrients. 1719;**2021**:13. DOI: 10.3390/ nu13051719