Aqueous extract of *Salvia officinalis* and *Ruta graveolens*: Potential source of reactive nitrogen species

Marwan S. M. Al-Nimer, Eham A. Ali[1]
Departments of Pharmacology and [1]Biochemistry, College of Medicine, Al-Mustansiriya University, Baghdad-Iraq

There are scarce evidences about the effects of herbs on nitrogen species that induced nitrosative stress. We here investigated the effect of simple water distilled extract of dry leaves *salvia officinalis* (sage) and *Ruta graveolens* (Rue) on the nitric oxide (NO) – peroxynitrite ($ONOO^-$) cycle biochemistry *in vitro* experiments. Aqueous extract of sage or rue (1%) were prepared by simple distillation and scanned by UV-visible spectrophotometer. Their effects were studied on the synthesized $ONOO^-$ as well as their ability to generate ONOO. It is ability to donate NO or to scavange released NO by sodium nitroprusside (10 mM) also investigated. UV-visible scan of sage extract revealed the presence of peaks at λ195 and 348.5 while that of rue extract at λ 200, λ242, λ291.5. Both extracts not generate $ONOO^-$ radical in form of nitrophenols. Rue extract increased the yield of prepared $ONOO^-$ by more than five times. Rue extract donated NO and improved the release NO from sodium nitroprusside while sage extract only improved the release of NO released by sodium nitroprusside. We conclude that simple distilled - aqueous extract of rue and sage extracts improved nitric oxide bioavailability that may be helpful in coronary artery disease with nitrate tolerance.

Key words: Nitric oxide, Peroxynitrite, *Ruta graveolens, Salvia officinalis*

INTRODUCTION

Salvia officinalis L. (sage) extracts had antioxidant activity *in vitro* and *in vivo* both in animals and humans.[1,2] Essential oil of sage reduced 2,2-diphenyl-1-picrylhydrazil (DPPH) and hydroxyl radicals formation (IC_{50} = 1.78 μg/ml) in dose dependent manner.[3] It slightly reduced nitric oxide (NO) synthesis.[4] *Ruta graveolens* L. (rue) extract showed dual effects on oxygen and nitrogen species. It scavenged the hydroxyl radicals at low concentrations but in higher concentrations it behaved as prooxidant accompanied with reducing ability to scavenge hydroxyl radical.[5] Raghav *et al,* found that high concentration of rue methanolic extract inhibited the expression of inducible nitric oxide synthase (iNOS) enzyme.[6]

Therefore, it is worth trial to investigate the effect of simple distilled water extract of dry leaves sage and rue on the nitric oxide – peroxynitrite cycle biochemistry *in vitro* experimental model.

MATERIALS AND METHODS

This study was conducted at Department of Pharmacology in cooperation with Department of Biochemistry, College of Medicine, Al-Mustansiriya University in Baghdad, Iraq during 2008.

Plant Material

Salvia officinalis leaves were obtained from Jordan and *Ruta graveolenes* leaves from Mosul in north of Iraq. Voucher specimens of the plants were taxonomically identified and deposited at the Department of Biology, College of Science, Al-Mustansiriya University in Baghdad, Iraq.

Extraction and Isolation

Aqueous extract of each was prepared by simple distillation. In brief, 1 g of each dried leaves in 100 ml distilled water (1%) was boiled, the vapor separated and condensed to obtain clear colourless liquid that was more concentrated in volatile compounds.

UV-visible spectra of distilled aqueous extract (1%) of each herb was recorded on a Aquarius (Cecil series with scanning ability, France) from 150–900 nm at room temperature with a 10 mm pathway length quartz cell with a scan rate of 600 nm/min.

Peroxynitrite Assay

Peroxynitrite ($ONOO^-$) was prepared by mixing 1 volume cooled hydrogen peroxide (1M) and 1 volume cooled sodium nitrite (1M) in dark room, then 2 volumes cooled NaOH (1.5M) was added to the mixture as prescribed by others.[7] Peroxynitrite ($ONOO^-$) was quantified spectrophotometrically (ε =

Address for correspondence: Dr. Marwan S. M. Al-Nimer, Department of Pharmacology, College of Medicine, Al-Mustansiriya University, PO Box 14132, Baghdad-Iraq. E-mail: alnimermarwan@ymail.com
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1670 $M^{-1}.cm^{-1}$). The yield was 1M. From this yield, 185μM $ONOO^-$ was incubated with 100 μl of each herbal extract in phosphate buffer saline for 15 minutes and the absorbance at 302 nm of the samples was recorded.

Peroxynitrite ($ONOO^-$) mediated nitration of phenol was measured for sage and rue extracts as described by others.[8,9] Briefly, 100 μl of 1% sage or rue extract was added to 5mM phenol in 50 mM sodium phosphate buffer pH 7.4 in a final volume of 3 ml. After incubation for 2 hours at 37°C, 50 μl 0.1M NaOH was added, and the absorbance at 412 nm of the samples was immediately recorded. The yield of nitrophenol was calculated from ε = 4400 $M^{-1}.cm^{-1}$. Further series of experiments were carried on by adding 100 μl exogenous $ONOO^-$(185 μM) to the each herb extract as above.

Nitric Oxide Assay

Nitric oxide donating activity was determined as described by Newaz and co-workers.[10] Briefly 500 μl of sage or rue extract (1%) was added to 50 μl HCl (6.5M) and 50 μl sulfunalic acid (37.5mM). After incubation for 10 min, 50 μl naphthylethylenediamine dihydrochloride (12.5mM) was added and incubated for further 30 min, centrifuged for 10 min at 1000g. The absorbance at 540 nm was immediately recorded. All experiments were performed in duplicate.

Nitric oxide radical scavenging was estimated by the use of Greiss reaction.[11] Greiss reagent was modified by using naphthylethylenediamine dihydrochloride (0.1% w/v). The reaction mixture was (3ml) containing sodium nitroprusside (10 mM, 2 ml), phosphate buffer (0.5 ml) and sage or rue extract (50–250 μl of 1%) or distilled water as negative control (final volume 0.5 ml) was incubated in dark room at 25°C for 150 min. After incubation, 0.5 ml of the reaction mixture mixed with 1 ml of sulfunalic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for complete diazotization. Then 1 ml of naphthylethylenediamine dihydrochloride was added, mixed and allowed to stand for 30 min in dark place at 25°C. A pink coloured chromophore is formed. The absorbance of these solutions was measured at 540 nm against corresponding blank. All experiments were performed in duplicate.

Chemicals and Drugs

Sodium nitroprusside and sodium nitrite purchased from Sigma-Aldrich, and chemicals used in the study were of Analr grade purchased from BDH. All the chemicals were prepared freshly prior to the experiment.

RESULTS

Figure 1 showed the presence of large peak at 195 nm and other small peak at 348.5 nm in UV-visible spectra of sage distilled water extract while rue extract showed three peaks at 200, 242 and 291.5 nm. The bioavailability of $ONOO^-$ as a result of non enzymatic reaction of hydrogen peroxide and sodium nitrite is improved in presence of rue extract by more than five times. It increased from baseline of 185 μmol to 995 μmol in presence of rue extract. This effect was not observed with sage extract.

Neither sage nor rue extracts released peroxynitrate by its own. Also there is no release of peroxynitrite radical detected at 412 nm for sage or rue extract when they are incubated with exogenous $ONOO^-$. Sage extract *per se* not released nitric oxide in reference to the positive control of sodium nitrite while rue extract released approximately 0.6 mmol.

Figure 2 showed that each herb improved NO bioavailability in presence of 10 mM sodium nitroprusside in a dose independent manner.

DISCUSSION

The results of the study suggest that both sage and rue

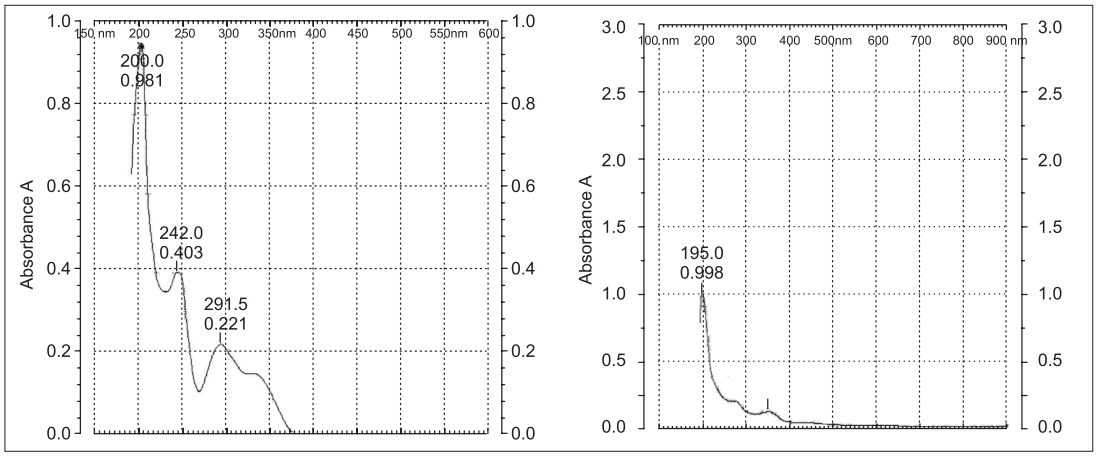

Figure 1: UV-Visible scan of (a) rue extract (b) sage extract

improved the nitric oxide bioavailability as a result of decomposition NO donor; sodium nitroprusside or sodium nitrite. Al least one of shared or not shared peaks in UV-visible scan is responsible for the biological activity of these herbs. Because the observed peaks in UV-visible scan are differed in sage compared with rue, the effects of these herbs on nitrogen species are also differed. In this study *S. officinalis* had no effect on $ONOO^-$. This finding is against the study of Kim *et al* who demonstrated $ONOO^-$ scavenging activity of red sage (*S. miltiorrhiza*).[12] Rue extract *per se* does not release ONOO. The interesting new finding is that rue extract potentiates the release of $ONOO^-$ from sodium nitrite. Most previous reports investigate the effect of herbs in scavenging rather than formation of ONOO.[13] Simply, the high level of $ONOO^-$ is attributed either to nitric oxide release by rue itself or it enhanced the non enzymatic reaction of sodium nitrite and hydrogen peroxide. The clinical potential of this effect is related to the cell growth inhibition and induction of apoptosis.[14] Therefore, this effect may explain the use of rue in management of brain tumour.[15] Previous studies demonstrated that some herbs released NO from cells.[16] and other herbs scavenged nitric oxide.[17] Oniga *et al*,[4] showed in *ex vivo* study that turpentine oil of saliva officinalis slightly reduced the nitric oxide synthesis *in vitro* test of phagocytosis of rat white blood cells. Also rue extract inhibits nitric oxide production in murine macrophage cells challenged with lipopolysaccharide as a result of inhibition of inducible nitric oxide synthase enzyme.[6]

Both rue and sage extracts improved the bioavailability of NO decomposed from sodium nitroprusside. The clinical significance of this finding linked to the inhibitory effect of NO on tumour cell growth.[18] Also these extracts are useful in patients with endothelial dysfunction treated with NO donors.

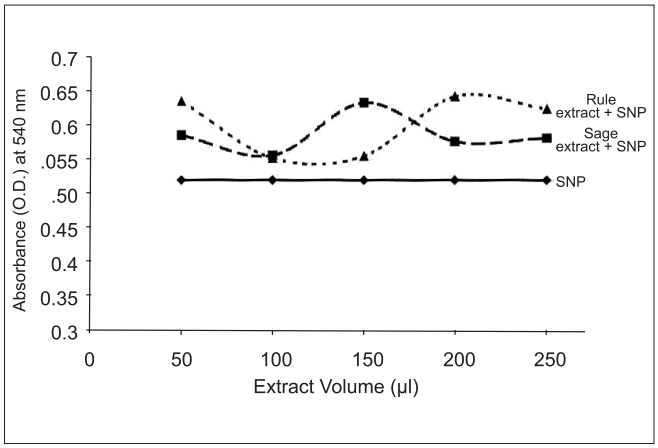

Figure 2: Effect of sage or rue extract on the nitric axide donated by sodium nitroprusside (SNP)

CONCLUSION

Simple aqueous distilled extracts of rue and to less extent the sage improved the bioavailability of nitric oxide that can be utilised in management of certain diseases like malignancies and endothelial dysfunction disorders.

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