International Journal of Drug Development and Research

Arumugam et al., Int J Drug Dev & Res 2015, 7:4

Research Article

# Modulatory Effects of *Mentha spicata* (Linn.) against 4 Nitroquinoline-1-Oxide Induced Chromosome Damage and Oxidative Stress in Mice

**Ponnan Arumugam[1*], Siva Kamalakannan[2] and Marudhamuthu Murugan[3]**

[1]*Department of Genetics, Dr. ALM Post Graduate Institute of Basic Medical Sciences, Taramani Campus, University of Madras, Chennai, Tamil Nadu, India*
[2]*Department of Zoology, School of Life Science, Bharathiar University, Coimbatore, Tamil Nadu, India*
[3]*Department of Microbial Technology, School of Biological Science, Madurai Kamaraj University, Madurai, Tamil Nadu, India*

***Corresponding author:** Dr. P. Arumugam, Department of Genetics, Dr. ALM Post Graduate Institute of Basic Medical Sciences, Taramani Campus, University of Madras, Chennai, Tamil Nadu, India, Tel: + 91 422 2428491; E-mail: ponnanarumugam@gmail.com

**Received** May 12, 2015; **Accepted** November 27, 2015; **Published** November 30, 2015

## Abstract

*Mentha spicata* is a common medicinal and edible plant in India and used in the ayurvedic system of medicine to treat various ailments including as a memory enhancer. The present study is aimed to evaluate the modulatory effects of *Mentha spicata* (Linn.) against 4-nitroquinoline-1-oxide (4-NQO) induced chromosome damage and oxidative stress in mice. Experiments were conducted with eight groups of either sex of mice. Ethanol extract (EE) of three doses (80, 160 and 320 mg/kg body weight-bw) with or without 4-NQO along with vehicle control (25% DMSO in water) were administered orally for five consecutive days. 4-NQO (7.5 mg/kg bw) was injected intraperitoneally on the sixth day, and the animals scarified the following day. 4-NQO enhanced the frequency of micronucleated polychromatic erythrocytes (MnPCEs) by about 4.2 times the control value, 15.78 MnPCEs/2500 PCEs. Pretreatment with EE, significantly reduced the MnPCEs frequency (50-69%) induced by 4-NQO. Moreover, 4-NQO enhanced the lipid peroxidation (LPO) by about 60% with decrease of enzymatic antioxidants in the range 27-41% and 38-60% for non-enzymatic antioxidants over the respective controls. Pre-treatment with the EE, brought down 4-NQO induced LPO significantly with in a dose dependent manner. All the measured antioxidants are positively modulated by the EE. The modulated values at high doses are either comparable with the controls or even higher than the control values.

**Keywords:** 4-NQO; Chromosome damage; Oxidative stress; Ethanol extract; *Mentha spicata*

**Abbreviations:** 4-NQO: 4-nitroquinoline-1-oxide; EE: Ethanol extract; DMSO: Dimethyl sulfoxide; MnPCEs: Micronucleated Polychromatic erythrocytes; NCEs: Normal chromatic erythrocytes; LPO: Lipid peroxidation; MDA: Malondialdehyde; DTNB: 5,5_-dithiobis (2-nitro benzoic acid); CDNB: 1-chloro 2,4-dinitrobenzene; GPx: Glutathione peroxidase; GST: Glutathione-s-transferase; SOD: Superoxide dismutase; CAT: Catalase; GSH: Reduced glutathione; G6PD: Glucose-6-phosphate dehydrogenase; BPO: Benzoxyl peroxide; ANOVA: Analysis of Variance; SNK Test: Student-Neuman-Keuls test; CPCSEA: Committee for the Purpose of Control and Supervision of Experiments on Animals

## Introduction

Oxidative stress is a major factor for the generation of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes, cancer, immunosuppression, neurodegenerative diseases etc. [1]. Many epidemiological and *in vitro* studies on medicinal plants and vegetables strongly supported that plant constituents with antioxidant activity are capable of exhibiting protective effects against the oxidative stress in biological systems [2]. Hence, research focused on the development of potential plant origin drugs for the elimination of mutagen/carcinogen induced health effects. Many plant extracts have been used as herbal drug for various toxins-induced illnesses in mammals, which have an advantage over the synthetic drug in terms of low or no toxicity at the effective dose [3]. Medicinal plants are also considered to be dietary substance due to their essential bioactive substances like vitamins, carotenoids, and flavonoids, glucoside, organic acids, sterols and some essential oils [4].

*Mentha spicata* (L.) belongs to the family Lamiaceae containing more than 200 genera and 5600 species [5]. It is also known as spearmint and as "Pudina" in India [6]. The genus Mentha has more than 25 species in which spearmint, peppermint, wild mint, corn mint, curled mint, bergamot, American mint and Korean mint are some well known species and among these spearmint is the most common [7]. The mint smell appears to stimulate the mind to feel for meat. Leaves are consumed in the form of herbal tea [8]. The leaves are used for making juice and chutneys usually in combination with other spices. Boiled leaf extract appears to relieve hiccup, flatulence, giddiness and enhance osteoarthritis symptoms [7,9]. It is also considered as stimulant, carminative and antispasmodic and a remedy for several diseases such as inflammation, fever, bronchitis, infantile troubles, vomiting in pregnancy and hysteria [10]. The plant is also known to have memory enhancing properties [11], antiplatelet [12], pests control [13], cytotoxic [14], chemopreventive [15], antioxidant [16,17] antimicrobial [18,19] and antifungal [20]. Recent reports revealed that *M. spicata* possessed a potent anti-inflammatory [21], radical scavenging [17] and anticancer properties [22]. Therefore, the present study was to evaluate the modulatory effects of *Mentha spicata* (Linn.) against 4-nitroquinoline-1-oxide induced chromosome damage and oxidative stress in mice.

## Materials and Methods

**Animals:** Swiss albino mice were obtained from the King Institute, Chennai, India. Both sex of mice (10-12 weeks old) and weighing 25-30 grams were used. Animals were maintained at the Institute's animal house under standard environmental conditions (temperature: 22 ± 2°C and 12 h light/dark period). Maintenance of animals was done in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. The Institute's ethical committee approved all experiments.

**Chemicals used in the study:** 4-Nitroquinoline-1-oxide (4-NQO), Malondialdehyde (MDA), Giemsa stain and May-Grunwald stain were purchased from Sigma-Aldrich, USA. Folin phenol reagent, 5,5_-dithiobis (2-nitro benzoic acid) (DTNB), 1-chloro

2,4-dinitrobenzene (CDNB) and reduced glutathione were purchased from SISCO Research Laboratories Pvt. Ltd. (Mumbai, India). All other solvents used in the experiments were of analytical grade.

### Plant material and extraction method

*M. spicata* L. was commercially purchased and identified at the Center for Advanced Studies in Botany, University of Madras (voucher number-855). Eight hundred grams of shade dried leaf powder was immersed in 4 L of 95% ethanol and left for 24 h under constant stirring and filtered. This was repeated twice with 2 L of 95% ethanol. Thus, a total of 6.5 L filtrate was collected and concentrated by rotary vapor at 40°C. The yield of ethanol extract (EE) was 42 g (5.25%). The extraction was done by the method of Villasenor et al. [23].

### Experimental design

Animals were divided into eight groups, with each group consisting of six mice of either sex. The concentrated ethanol leaf extract was dissolved in 25% DMSO and administered by oral gavage to the mice for 5 consecutive days. Group 1, the control, was administered 25% DMSO. Groups 2, 3, and 4 received doses of 80, 160, and 320 mg/kg bwt extract, respectively. Group 5 was administered 4-NQO alone (7.5 mg/kg bwt intraperitoneally; i.p.). Groups 6, 7, and 8 received the doses of 80, 160, and 320 mg/kg bwt of ethanol extract for 5 days, and after 2 hours, 4-NQO (7. 5 mg/kg bwt) was injected. After 24 hours of 4-NQO treatment, all the animals were sacrificed by cervical dislocation. The mouse bone marrow assay was carried out by the method of Schmid which was previously published [24]. For each animal (experimental/control), 2,500 polychromatic erythrocytes (with or without micronuclei) and a corresponding number of normal chromatic erythrocytes (NCEs) were scored under a light microscope. Mouse liver was immediately excised and store in the physiological saline until use.

### Bio-chemical assays estimated by following conventional methods

Homogenate (10%) was prepared in 0.1 M Tris-HCl buffer (pH 7.4), using a Potter-Elvehjem homogenizer with a Teflon pestle. The homogenate was used in bioassays of total protein [25], LPO [26], glutathione peroxidase [27], glutathione-s-transferase (GST) [28], superoxide dismutase (SOD) [29], catalase (CAT) [30], reduced glutathione (GSH) [31], Vitamin E [32] and Vitamin C [33] estimated using conventional procedures.

### Statistical analysis

Results were presented as mean ± standard error for six mice of each group. Statistical analyses were performed by one-way ANOVA using SPSS Software Version 12.0. The Student-Neuman-Keuls test (SNK TEST) was applied to assess differences among the groups. Values of $P \leq 0.05$ were considered to be significant.

## Results

### Modulated 4-NQO induced genetic damage by *M. specata*

The results on the frequency of micronucleated polychromatic erythrocytes (MnPCEs) for the various treatment groups are presented in Tables 1 and 2. 4-NQO, enhanced the frequency of MnPCEs by about 4.2 times the control value, 15.78 MnPCEs/2500 PCEs. Irrespective of the dose, treatment with the EE alone had shown no significant effect on the frequency of MnPCEs. However, pre-treatment with EE, significantly reduced the mutation frequency induced by 4-NQO. The reduction was greatest at the highest dose, 320 mg/Kg bwt (by about 69%) and least at the lowest dose, 80 mg/Kg bwt (about 50%). However, the effects by different doses were not consistently significant (P<0.05).

### Modulated 4-NQO altered LPO by *M. specata*

4-NQO enhanced the LPO levels by about 60 percent over the control value, 1.84 nmoles of MDA formed/mg protein. EE treatment alone had not shown any effect on the LPO. Pre-treatment with the EE, brought down 4-NQO induced LPO significantly at all the doses tested. At dose 320 mg/Kg bwt, the reduction was effective and comparable with the control value. The reduction of LPO was dose dependent (Figure 1).

### Modulated 4-NQO altered enzymatic antioxidants by *M. specata*

4-NQO significantly affected the values of enzymatic antioxidants (GPx, GST, SOD and CAT) to an extent of 27 and 41% over their control values (group 1 vs 5, *P*<0.05, Table 1). Among them, GST was the most affected enzyme. The mutagen affected the enzyme value by about 41% of the control value. Both GPx and SOD were equally affected (~38 and 37%, respectively) by 4-NQO. Treatment with EE alone had shown no effect on any of these enzymes. The levels of all enzymes were restored to their control level with the pretreatment of EE. However, the dose response of recovery varied with the enzymes. The most affected enzyme, GST was restored to the control level only at doses, 160 and 320 mg/Kg bwt. At the lowest dose, 80 mg/Kg bwt, though significant recovery was observed, the enzyme level was not comparable with the control value (P<0.05, Table 1). The recovery of CAT was more dramatic. The lowest dose was not effective (80 mg/Kg bwt; group 5=6, P>0.05). But the dose, 160 mg/Kg bwt itself was most effective. The recovery of SOD was dose dependent. The recovery was effective at the dose of 160 mg/Kg bwt. On the other hand, the recovery of GPx was effective, even at the lowest dose (80 mg/Kg bwt).

### Modulated 4-NQO altered non-enzymatic antioxidants by *M. specata*

Unlike enzymatic antioxidants, the three non-enzymatic antioxidants viz., GSH, Vitamin E and Vitamin C were the most affected (Figure 1). The range of decrease of the non-enzymatic antioxidants was much higher (38-60%), compared to the range of decrease, (27-41%) observed for the enzymatic antioxidants. Of the three non-enzymatic antioxidants, the GSH decreased to the extent of 60% of the control value, 1.54 µg/mg protein followed by Vitamin E (47%). Their recovery following the pretreatment with EE varied. The GSH recovery was effective even at the lowest dose, 80 mg/kg bwt. In fact, the values of this antioxidant were significantly higher than the control

| Treatment (Dose: mg/kg bwt) | MnPCEs/2500 PCEs | PCE/NCE | % Decrease over 4-NQO induced MnPCEs |
|---|---|---|---|
| Control | 15.78 ± 0.87 | 1.41 | – |
| EE (320) | 16.55 ± 1.96 | 1.13 | – |
| 4-NQO (7.5) | 67.12 ± 4.93$^{a***}$ | 0.75 | 100.00 |
| EE (80)+4-NQO (7.5) | 33.37 ± 3.00$^{b***}$ | 1.18 | 50.30 |
| EE (160)+4-NQO (7.5) | 27.28 ± 1.90$^{b***}$ | 1.11 | 59.40 |
| EE (320)+4-NQO (7.5) | 21.13 ± 2.60$^{b***}$ | 1.25 | 68.90 |

EE: Ethanol Extract; Mean ± Standard Error (*n*=6); 4-NQO: 4-nitroquinoline-1-oxide; 2500 PCEs/mice; MnPCEs: micronucleated polychromatic erythrocytes; NCE: normalchromatic erythrocytes; significant difference at ****P*<0.001 and **P*<0.05 (Student-Newman-Keuls), 'a' stand for comparison with control group, 'b' stand for comparison with 4-NQO alone group.

**Table 1:** Modulation of 4-NQO induced chromosome damage in mice pretreated with ethanol extract of *Mentha spicata*.

| Treatment (Dose: mg/kg bwt) | GPX | GST | SOD | CAT |
|---|---|---|---|---|
| Control | 43.41 ± 2.42 | 2.55 ± 0.03 | 5.36 ± 0.29 | 343.35 ± 16.04 |
| EE (320) | 48.17 ± 2.73 | 2.67 ± 0.08 | 5.29 ± 0.08 | 347.53 ± 17.07 |
| 4-NQO (7.5) | 27.02 ± 1.66$^{a***}$ | 1.51 ± 0.03$^{a***}$ | 3.36 ± 0.17$^{a***}$ | 249.22 ± 15.32$^{a***}$ |
| EE (80) + 4-NQO (7.5) | 36.39 ± 2.88$^{b*}$ | 1.85 ± 0.05$^{b*}$ | 4.59 ± 0.16$^{b*}$ | 266.84 ± 8.31$^{b\#}$ |
| EE (160) +4-NQO (7.5) | 44.39 ± 2.02$^{b***}$ | 2.89 ± 0.04$^{b***}$ | 5.54 ± 0.11$^{b***}$ | 323.83 ± 19.75$^{b***}$ |
| EE (320) +4-NQO (7.5) | 49.96 ± 2.36$^{b***}$ | 2.61 ± 0.02$^{b***}$ | 6.48 ± 0.07$^{b***}$ | 356.63 ± 21.27$^{b***}$ |

EE: ethanol extract; Mean ± standard error ($n$=6); Significant difference at ***$P$<0.001 and *$P$<0.05; "a" represents the comparison between the control vs. the 4-NQO; "b" represents the comparison between the 4-NQO vs. the EE+4-NQO; "#" not significant; GPx: mol of reduced glutathione oxidized/min/mg protein; GST: nmol of CDNB-conjugated/min/mg protein; SOD: units/min/mg protein; CAT: µmol of $H_2O_2$ consumed/min/mg protein.

**Table 2:** Modulatory effect of ethanol extract of *Mentha spicata* pretreatment on 4-NQO induced changes in enzymatic antioxidants in mice.

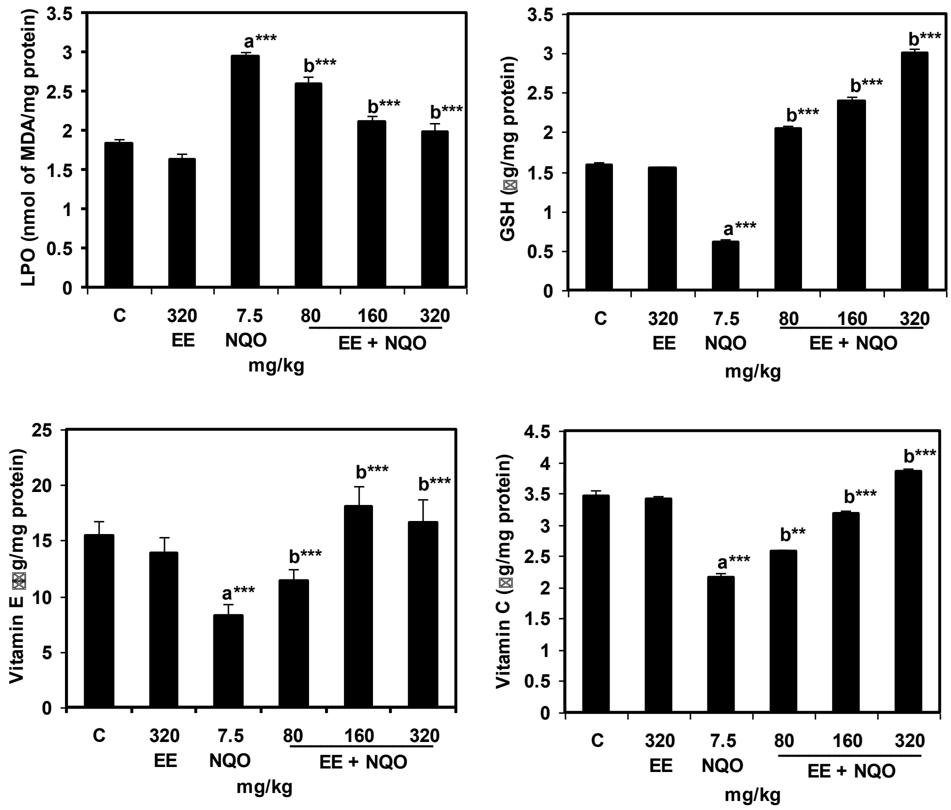


**Figure 1:** Modulatory effect of ethanol extract of *Mentha spicata* pretreatment on 4-NQO induced changes in LPO and liver non-enzymatic antioxidants in mice. (Mean ± standard error ($n$=6); significant difference at ***$P$<0.001 and *$P$<0.05; "a" represents the comparison between control vs. the 4-NQO; "b" represents the comparison between the 4-NQO vs. the AF+4-NQO groups.

values at doses 160 and 320 mg/Kg bwt. The recovery of Vitamin E was effective only at doses, 160 and 320 mg/Kg bwt. Vitamin C recovery was entirely dose dependent and was effective at the highest dose alone. Thus, LPO induced by 4-NQO was modulated by ethanol extract of *M. spicata* shade dried leaves by effective restoration of non-enzymatic antioxidants in particular that of GSH.

## Discussion

Reactive oxygen species (ROS) are increasingly produced in animals treated with mutagens [34]. 4-NQO was reported to generate LPO through the formation of superoxide anion and hydrogen peroxide during its metabolism [35]. Thus, overload of ROS leads to oxidative stress by damaging macromolecules [36]. The present study evaluated 4-nitroquinoline-1-oxide (4-NQO) induced chromosome damage and oxidative stress in mice modulated by EE obtained from leaves of *M. spicata*. 4-NQO enhanced the frequency of MnPCEs by about 4.2 times the control value, 15.78 MnPCEs/2500 PCEs. Treatment with EE effectively decreased the 4-NQO enhanced MnPCE frequency by about 50% to 69%, depending on dose. The maximum amount of reduction i.e., 69% was observed at a dose of 320 mg/Kg bwt. PCE/NCE ratio also significantly decreased by about 0.75 in 4-NQO-alone group when compared to the control value, 1.41. Pretreatment with EE increased the PCE/NCE ratio in the range between 1.11 and 1.25, which was near to the control level. Prior report also demonstrated that *Mentha* extracts possessed tremendous activity against various mutagens/carcinogens [37].

LPO increased in biological system due to the overload of oxidative stress and insufficient defense against the mutagen exposure [38]. 4-NQO enhanced LPO by about 60% over the control value, i.e., 1.84 nmoles of MDA formed/mg protein (Figure 1). EE effectively suppressed 4-NOQ enhanced LPO. Reduction was significant although it was not always to the level observed in control groups. Moreover, biological antioxidants primarily restrict the deleterious effects of oxidative stress and their decrease reflects saturation with ROS which in turn may lead to cellular death [39]. 4-NQO affected all *in vivo* antioxidants measured (Table 1 and Figure 1). The decrease in the enzymatic antioxidants was in the range 27-41% and for non-enzymatic antioxidants in the range 38-60%. The most affected antioxidant was GSH (decreased by ~ 60%) and the least affected was CAT (decreased by ~ 28%). EE positively modulated all the measured antioxidants. The magnitude of modulation varied with dose. The modulated values at high doses are either comparable with the controls or even higher than the control values. *Mentha* extract showed protective effect against gamma radiation exposed to mice by elevating GSH and decreasing LPO [40]. Saleem et al. [15] explored the modulating effect of spearmint extract on benzoxyl peroxide (BPO) altered LPO and antioxidant levels in mice. They observed a significant decrease in LPO and raise in GSH, CAT, G6PD, GPx, GR and GST levels. Similarly, EE of aromatic plants showed protective effects against D-galactose induced oxidative stress in mice [41]. Other experiments supported that the feeding of medicinal plants extracts in rat results in an increase of antioxidant enzyme activity [3].

The mechanism of antioxidant is essential, SOD converts two superoxide anions into an oxygen and hydrogen peroxide molecules. CAT breaks down the $H_2O_2$ into oxygen and water. Alternatively, GPx detoxifies the $H_2O_2$ and hydroxyl radicals. The latter is more important than the catalase in removing $H_2O_2$. GPx also has the ability to react with lipid peroxides [42]. The mutagen 4-NQO can be detoxified by GST with GSH conjugation to give 4 (glutathione-s-yl) quinoline-1-oxides and protect cells from the carcinogenic agents [35]. The present study also revealed that the non-enzymatic antioxidant levels were better than control values (Figure 1). The activities of non-enzymatic antioxidants viz., Vitamin E, Vitamin C and GSH were consistent in metabolisation of mutagens [43]. Vitamin E scavenges the peroxyl and alkoxyl radicals by donating one electron and in turn become tocopheroxyl radical. Vitamin C recycles these radicals back to Vitamin E [44]. The oxidized ascorbic acid (dehydroascorbate) is reduced to ascorbic acid by GSH. Thus, GSH is the primary antioxidant in defense against various environmental toxic chemicals [45]. The present results exhibited that GSH is effective even at the lowest dose, 80 mg/kg bwt and their values were significantly higher than the control. Vitamin E was effective only at doses, 160 mg/kg bwt whereas vitamin C was entirely dose dependent. Yao et al. [46] also reported that *Ginkgo biloba* extract reduced LPO by enhancing GSH activity in oxidative stress induced rat liver by ethanol.

It is well know that *M. spicata* supported can be used as natural antioxidant because it various biological properties. Since from ancient, it is commonly used edible and medicinal plants in India. Based on the scientific knowledge and the present work clearly revealed the 4-NQO induced chromosome damage with oxidative stress in mice was effectively modulated by ethanol extract of *M. spicata* leaves due to the presence of synergetic secondary metabolites.

### Acknowledgments

This work funded by University Grant Commission (UGC) was gratefully acknowledged.

### Conflict of Interest

The authors declare that there was no conflict of interest in this current publication.

### References

1. Jayasri MA, Mathew L, Radha A (2009) A report on the antioxidant activity of leaves and rhizome of Costus pictus D. Don IJIB 5: 20-26.
2. Souri E, Amin G, Farsam H, Jalalizadeh H, Barezi S (2008) Screening of Thirteen Medicinal Plant Extracts for Antioxidant Activity. IJPR 7: 149-154.
3. Jastrzebski Z, Medina OJ, Moreno LM, Gorinstein S (2007) In vitro studies of polyphenol compounds, total antioxidant capacity and other dietary indices in a mixture of plants (Prolipid). Int J Food Sci Nutr 58: 531-541.
4. Saggu S, Kumar R (2008) Effect of seabuckthorn leaf extracts on circulating energy fuels, lipid peroxidation and antioxidant parameters in rats during exposure to cold, hypoxia and restraint (C-H-R) stress and post stress recovery. Phytomedicine 15: 437-446.
5. Hedge C (1992) A global survey of the biogeography of the Labiatae. In Harely RM & Reynolds T (eds) Advances in Labiatae Science. Royal Botanic Gardens. Kew: 7-17.
6. Shaiq Ali M, Ahmed W, Saleem M, Khan T (2006) Longifoamide-A and B: Two new ceramides from Mentha longifolia (Lamiaceae). Nat Prod Res 20: 953-960.
7. Choudhury RP, Kumar A, Garg AN (2006) Analysis of Indian mint (Mentha spicata) for essential, trace and toxic elements and its antioxidant behaviour. J Pharm Biomed Anal 41: 825-832.
8. Carmona MD, Llorach R, Obon C, Rivera D (2005) “Zahraa”, a Unani multicomponent herbal tea widely consumed in Syria: components of drug mixtures and alleged medicinal properties. J Ethnopharmacol 102: 344-350.
9. Connelly AE, Tucker AJ, Tulk H, Catapang M, Chapman L, et al. (2014) High-rosmarinic acid spearmint tea in the management of knee osteoarthritis symptoms. J Med Food 17: 1361-1367.
10. Kanatt SR, Chander R, Sharma A (2007) Antioxidant potential of mint (Mentha spicata L.) in radiation- processed lamb meat. Food Chem 100: 451-458.
11. Adsersen A, Gauguin B, Gudiksen L, Jäger AK (2006) Screening of plants used in Danish folk medicine to treat memory dysfunction for acetylcholinesterase inhibitory activity. J Ethnopharmacol 104: 418-422.
12. Tognolini M, Barocelli E, Ballabeni V, Bruni R, Bianchi A, et al. (2006) Comparative screening of plant essential oils: phenylpropanoid moiety as basic core for antiplatelet activity. Life Sci 78: 1419-1432.
13. Eliopoulos PA, Hassiotis CN, Andreadis SS, Porichi AE (2015) Fumigant Toxicity of Essential Oils from Basil and Spearmint Against Two Major Pyralid Pests of Stored Products. J Econ Entomol 108: 805-810.
14. Manosroi J, Dhumtanom P, Manosroi A (2006) Anti-proliferative activity of essential oil extracted from Thai medicinal plants on KB and P388 cell lines. Cancer Lett 235: 114-120.
15. Saleem M, Alam A, Sultana S (2000) Attenuation of benzoyl peroxide-mediated cutaneous oxidative stress and hyperproliferative response by the prophylactic treatment of mice with spearmint (Mentha spicata). Food Chem Toxicol 38: 939-948.
16. Snoussi M, Noumi E, Trabelsi N, Flamini G, Papetti A, et al. (2015) Mentha spicata essential oil: chemical composition, antioxidant and antibacterial activities against planktonic and biofilm cultures of vibrio spp. strains. Molecules. 20: 14402-14424.
17. Arumugam P, Murugan R, Subathra M, Ramesh A (2010a) Superoxide radical scavenging and antibacterial activities of different fractions of ethanol extract of Mentha spicata (L). Med Chem Res 32: 411-416.
18. Ullah N, Khurram M, Amin MU, Afridi HH, Ali Khan F, et al. (2011) Comparison of phytochemical constituents and antimicrobial activities of Mentha spicata from four northern districts of Khyber pakhtunkhwa. J Appl Pharmaceut Sci 01: 72-76.
19. Shahbazi Y (2015) Chemical Composition and In Vitro Antibacterial Activity of Mentha spicata Essential Oil against Common Food-Borne Pathogenic Bacteria. J Pathog 2015: 916305.
20. Kedia A, Dwivedy AK, Jha DK, Dubey NK (2015) Efficacy of Mentha spicata essential oil in suppression of Aspergillus flavus and aflatoxin contamination in chickpea with particular emphasis to mode of antifungal action. Protoplasma.
21. Arumugam P, Priya NG, Subathra M, Ramesh A (2008) Anti-inflammatory activity of four solvent fractions of ethanol extract of Mentha spicata L. investigated on acute and chronic inflammation induced rats. Environ Toxicol Pharmacol 26: 92-95.
22. Sharma V, Hussain S, Gupta M, Saxena AK (2014) In vitro anticancer activity of extracts of Mentha Spp. against human cancer cells. Indian J Biochem Biophys 51: 416-419.

23. Villaseñor IM, Echegoyen DE, Angelada JS (2002) A new antimutagen from Mentha cordifolia Opiz. Mutat Res 515: 141-146.

24. Arumugam P, Ramesh A (2009) Protective effects of solvent fractions of Mentha spicata (L.) leaves evaluated on 4-nitroquinoline-1-oxide induced chromosome damage and apoptosis in mice bone marrow cells. Genet Mol Biol 32: 847-852.

25. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265-275.

26. Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95: 351-358.

27. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, et al. (1973) Selenium: biochemical role as a component of glutathione peroxidase. Science 179: 588-590.

28. Habig WH, Pabst MJ, Jakoby WB (1974) Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem 249: 7130-7139.

29. Marklund S, Marklund G (1974) Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem 47: 469-474.

30. Sinha AK (1972) Colorimetric assay of catalase. Anal Biochem 47: 389-394.

31. Moron MS, Depierre JW, Mannervik B (1979) Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochim Biophys Acta 582: 67-78.

32. Desai ID (1984) Vitamin E analysis methods for animal tissues. Methods Enzymol 105: 138-147.

33. Omaye ST, Turnbull JD, Sauberlich HE (1979) Selected methods for the determination of ascorbic acid in animal cells, tissues, and fluids. Methods Enzymol 62: 3-11.

34. Li HB, Wong CC, Cheng KW, Chen F (2008) Antioxidant properties in vitro and total phenolic contents in methanol extracts from medicinal plants. LWT 41: 385-390.

35. Kanojia D, Vaidya MM (2006) 4-Nitroquinoline-1-oxide induced experimental oral carcinogenesis. Oral Oncol 49: 3056-3059.

36. Diekmann M, Waldmann P, Schnurstein A, Grummt T, Braunbeck T, et al. (2004) On the relevance of genotoxicity for fish populations II: genotoxic effects in zebrafish (Danio rerio) exposed to 4-nitroquinoline-1-oxide in a complete life-cycle test. Aquat Toxicol 68: 27-37.

37. Yu TW, Xu M, Dashwood RH (2004) Antimutagenic activity of spearmint. Environ Mol Mutagen 44: 387-393.

38. Srinivasan P, Sabitha KE, Shyamaladevi CS (2007) Attenuation of 4-nitroquinoline 1-oxide induced in vitro lipid peroxidation by green tea polyphenols. Life Sci 80: 1080-1086.

39. Koul A, Bhatia V, Bansal MP (2001) Effect of alpha-tocopherol on pulmonary antioxidant defence system and lipid peroxidation in cigarette smoke inhaling mice. BMC Biochem 2: 14.

40. Samarth RM, Kumar A (2003) Mentha piperita (Linn.) leaf extract provides protection against radiation induced chromosomal damage in bone marrow of mice. Indian J Exp Biol 41: 229-237.

41. Patil RB, Vora SR, Pillai MM (2009) Antioxidant effect of plant extracts on phospholipids levels in oxidatively stressed male reproductive organs in mice IJRM 7: 35-39.

42. Sreelatha S, Padma PR, Umadevi M (2009) Protective effects of Coriandrum sativum extracts on carbon tetrachloride-induced hepatotoxicity in rats. Food Chem Toxicol 47: 702-708.

43. Kim KS, Lee S, Lee YS, Jung SH, Park Y, et al. (2003) Anti-oxidant activities of the extracts from the herbs of Artemisia apiacea. J Ethnopharmacol 85: 69-72.

44. Chanwitheesuk A, Teerawutgulrag A, Rakariyatham N (2005) Screening of antioxidant activity and antioxidant compounds of some edible plants of Thailand. Food Chem 92: 491-497.

45. Farooq SM, Asokan D, Sakthivel R, Kalaiselvi P, Varalakshmi P (2004) Salubrious effect of C-phycocyanin against oxalate-mediated renal cell injury. Clin Chim Acta 348: 199-205.

46. Yao P, Li K, Song F, Zhou S, Sun X, et al. (2007) Heme xygenase-1 upregulated by Ginkgo biloba extract: Potential protection against ethanol-induced oxidative liver damage. Food Chem Toxicol 45: 1333-1342.