The mutagenic activity of N
...oral beverage or a herbal remedy to provide means of protection against cancer. This approach goes hand in hand becomes of great importance to search for a simple diet, a comm...ings. The PAHs are listed as major compositions in peppermint. However, no study has been reported the ...fective. Hence, the objective of the study was to evaluate the ant carcinogenic effect of Madinah mint plant leaves.

The polycyclic aromatic hydrocarbon” (PAH) refers to organic compounds that consist of two or more fused aromatic rings. The PAHs are listed as carcinogens including, Benz[a]anthracene, benzo[a]pyrene, dibenz[a,h]anthracene, dibenz[a,h]pyrene and dibenz[a]pyrene. Evidence for their carcinogenicity from studies in experimental animals was reported [Khan et al., 2012a].

Since human cancer occurrence is linked to dietary habits and lifestyle, as clearly indicated by mounting evidence, it becomes of great importance to search for a simple diet, a common beverage or a herbal remedy to provide means of protection against cancer. This approach goes hand-in-hand with the increasing attention that has recently focused on the use of cancer chemotherapeutic agents for individuals at high risk of neoplastic development [Khan et al., 2012b].

Related studies have demonstrated that Mint extract is a potent antioxidant, antimicrobial activity and anti-insecticide [Karakas et al., 2000]. The present study was undertaken in an attempt to assess whether boiling water mint extract (BWME) modulates the cytochrome P450 mixed function oxidase system, as well as phase II enzymes, by given rats mint extract in drinking water.

Methods
Mint extraction

Peppermint (M. piperita L.) was purchased from Madinah, KSA. The specimens were identified by stuff member, Department of biological science, KAU, Jeddah. The only green leaves were washed with distilled water and kept at 4 °C until used. One hundred grams of mint leaves was boiled in one liter distal water to obtain (10%, W/V)
Animals

The handling of animals was done according to the ethical committee of KAU. Male Wister albino rats (100-120 g) were purchased from the animal house in the King Fahd Research Center, King Abdulaziz University, Jeddah, KSA. The rats were randomly divided into two groups, comprising 12 animals each. The first group served as control, whereas the second was maintained on BWME (10% w/v) as its sole drinking liquid for six weeks. The animals were then decapitated after anaesthesia by thiopental.

Preparation of hepatic microsomal fractions

The liver homogenate and subcellular fractions was achieved according to the method of Ioannides and Parke [11]. Liver was homogenized using glass homogenizer with 25% (w/v) suspension with ice-cold 1.15% (w/v) KCL. The homogenate was centrifuged at 10000g for 15 min at 4°C using cooling centrifuge. The supernatant, microsomal supernatant was stored at -20°C until use. For the preparation of microsomal were centrifuged at 55,000 rpm for 60 min at 4°C. The pellet was re-suspended in the original volume, of 1.15% (w/v) KCL. The re-suspended pellet is termed the microsomal fraction.

Enzyme assays

The protein levels of the hepatic microsomes was measured by the Lowry procedure [12], using bovine serum albumin as a standard. The following enzyme assays were carried out using isolated microsomes: Ethoxyresorufin O-deethylase [Khan et al., 2012a], methoxyresorufin O-demethylase [Khan et al., 2013], p-nitrophenol hydroxylase [Khan et al., 2012b], erythromycin N-demethylase [Castillo-Juarez et al., 2009], epoxide hydrolase using benzo(a)pyrene-4,5-oxide as substrate [Liang et al., 2012] and UDP-glucuronosyltransferase, employing 2-amino-phenol as a substrate, [Lopez et al., 2010]. In addition to these microsomal assays, the following enzyme activities were performed on the cytosolic fraction: Glutathione S-transferase [Stafford et al., 2005], and sulphotransferase using 2-naphthol as a substrate [Lucchesi et al., 2004].

Mutagenicity assays

The effect of treatment with aqueous mint extract on the activation of model mutagens was determined using the Ames Salmonella test [Hussain et al., 2010]. The activation system was prepared from control and mint-treated animals. All mutagens (benzo(a)pyrene 25µg, dimethylbenz[a]anthracene 50ug, and N-nitrosopyrolidine 6 mg) were dissolved in DMSO, so that the final concentration remained below 100 µl per incubation.

Statistical analysis

The data were analyzed by using the SPSS software. The student’s t-test was used to examine the statistical significance of differences among the means of different treatments within this Complete Randomized Design. The significant differences are reported at P value of < 0.05.

Results

Results in Table 1, shows that, the treatment of rats with BWME impaired the O-dealkylations of methoxy- and ethoxyresorufin. Similarly, there was a significant decrease in the O-depentylation of pentoxyresorufin (p<0.01) together with the demethylation of methoxyresorufin (p<0.01) whereas only a significant decrease in the hydroxylation of p-nitrophenol (p<0.01) was determined. In contrast, there was a significant elevation of erythromycin N-demethylase (p<0.01) in rats depend on mint extract as compared with control group.

Table 1: Effect of BWME on liver mixed function oxidases activities (mean ± SD)

| Enzyme activity                  | Control N=12 | BWME N=12 |
|----------------------------------|--------------|-----------|
| Methoxyresorufin O-demethylase    |              |           |
| (Unit /mg protein )              | 41.8±5.6     | 22.3±4.7**|
| Pentoxysorufin O-depentylation    | 13.6±2.2     | 6.6±0.3** |
| (Unit /mg protein )              |              |           |
| Ethoxyresorufin O-deethylase      | 58.9±6.9     | 11±3**    |
| (Unit /mg protein )              |              |           |
p-Nitrophenol hydroxylase (Unit/mg protein)  
24.6 ± 2.4  
10.3 ± 0.77**

Erythromycin N-demethylase (Unit/mg protein)  
18 ± 1.3  
47.6 ± 8.1**

*P < 0.05, **P < 0.01

Effect of BWME on rat hepatic conjugation enzyme activities

The effect of mint extract administration on liver phase II detoxifying enzymes in Table 2 showed that, mint extract caused a significant elevation in the activity of epoxide hydrolase (p<0.001), as compared with control. However, glutathione S-transferase and UDP-glucuronosyltransferase activities were significantly decreased (p<0.001, p<0.01) respectively following treatment with mint extract. No significant change was recorded in the activity of sulphotransferase, which was monitored using 2-naphthol substrate on when measured in the presence of 2-aminophenol.

Table 2: Effect of BWME extract on rat hepatic phase II enzyme activities (mean ± SD).

| Parameter                        | Control (N=12) | Mint extract (N=12) |
|----------------------------------|----------------|---------------------|
| Epoxide hydrolase (unit/mg protein) | 18.5 ± 2.3     | 27.9 ± 3.4          |
| Glucuronosyl transferase (unit/mg protein) | 46.6 ± 6.9    | 33.5 ± 4.6          |
| Glutathione S-transferase (unit/mg protein) | 55.8 ± 3.3   | 31.0 ± 4.4          |
| Sulphotransferase (unit/mg protein) | 12.3 ± 1.1     | 11.2 ± 2.1          |

*P < 0.05, **P < 0.01

Effect of BWME treatment on the bio-activation of chemical carcinogens

Liver microsomal preparations from mint-treated rats were less effective in bio activating benzo(a)pyrene, compared with control (p<0.001) (Figure 1), and a similar picture was obtained 7,12 di methylbenzo (a)anthracene at different concentration in the Ames test. The mutagenic activity of N-nitrosopiperidine was lower in the mint-treated hepatic microsomal compared with controls (Figure 3) and N- nitrosopyrrolidine (Figure 4).

Figure 1: Effect of BWME drinking on bio activation of benzo(a)pyrene in liver rats.
Figure 2: Effect of BWME drinking on bioactivation of 7,12 dimethyl benz(a)anthracene in liver rats.

Figure 3: Effect of mint drinking on bioactivation of N-nitrosopiperidine in liver rats.

Figure 4: Effect of mint drinking on bioactivation of N-nitrosopyrroldine in liver rats.
Discussion

Several dietary natural products have the ability to modulate the process of carcinogenesis in experimental animal models. This modulation mediated by their effect on detoxifying enzyme systems that catalyze the metabolic activation or deactivation of chemical carcinogens [McKay et al., 2006]. The present study was concerned with the effects of BWME administration on hepatic carcinogen-metabolizing enzymes (phase I and II). It was found that, treatment with BWME for six weeks significantly decreased the activity the O-demethylation of methoxyresorufin, which is a selective probe for CYP1A2 activity [Shimada et al., 1992]. In addition BWME showed to reduce the activity of O-deethylation of the ethoxyresorufin, which operates as a diagnostic probe for CYP1A1 activity, although it is also metabolized by CYP1A2 [Wadsworth et al., 2001]. Similarly, administration of BWME caused a marked decrease in the O-depentylation of pentoxyresorufin, which is selectively catalyzed by the CYP2B subfamily [Horn and Vargas, 2003]. There is a significant decrease in the hydroxylation of p-nitrophenol, which is catalyzed by CYP2E following to BWME for six weeks [Ga Ayoola et al., 2008]. Inversely, the N-demethylation of ethrythromycin, a marker of CYP3A activity [Gulluce et al., 2007] was significantly increased in rats taking BWME compared with control.

From this results we can deduce that treatment of rats with BWME caused a decrease in hepatic cytochrome P450 proteins, such as CYP2B, CYP3E1 and CYP1A, the latter P450 sub-family being closely associated with inhibiting bioactivation of chemical carcinogens. The ability of BWME in this carcinogen modulation may be related to its active ingredients contents volatile oils as menthol as reported by Khan and Abourashed [Hideyuki et al., 2002], who reported that peppermint yields up to 1.0 % of volatile oils including menthol, menthone and menthyl acetate. It was reported that, (benzo (a)pyrene and 7,12 dimethylbenzanthracene) are both promutagens. The CYP1A1 and CYP2B activities that metabolize these PAH need liver microsomes for bio-activation and enhanced by BWME. It was also related to the decrease in CYP1A1 and CYP2B activities compared with control.

The mutagenicity of nitrosopiperidine and nitrosopyrrolidine, nitrosoamines selectively activated by CYP2E, was not influenced by This may be explained by the fact that constituents of mint as menthol can impair the activity of CYP2E. Conversely, it may be inferred that the components of the hepatic preparation from BWME-treated rats, inhibit the formation and/or scavenges the reactive intermediate of both carcinogens. It was suggested that BWME may possess anti-carcinogenic potential, by directing the metabolism of the chemical carcinogens in such a way as to favor their detoxification. The present study was therefore also undertaken to establish whether the decrease in mixed-function oxidase activities and carcinogen activation is accompanied by changes in the phase II enzymes. Glutathione conjugation is probably the most effective metabolic pathway for eliminating reactive intermediates, a process which is catalyzed by glutathione S-transferase [Hammons et al., 1999]. Such mechanism of action, however, appeared unlikely, since the measured activity of glutathione S-transferase was unaffected by the treatment with BWME. The same treatment with BWME also failed to modulate sulphotransferase conjugation, and microsomal epoxide hydrolase activity, in rat liver. Similarly, after pre-treatment of rats with BWME, there was no observable increase in the glucuronidation of 2-amino phenol activity.

However, the observed results appeared unlikely, since there was no marked increase in the observed levels of the enzyme activity, following the BWME administration. Mint extract may also exert its anti-carcinogenic potential by stimulating the enzyme system that deactivates reactive oxygen species.

Conclusion

The present study has shown that BWME has the potential to suppress the activity of cytochrome enzymes involved in the bio-activation of chemical carcinogen and, consequently, may display chemo preventive activity.

Acknowledgment

This work was funded by the Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah, under grant no (RG-9-130-37). The authors, therefore, acknowledge with thanks DSR technical and financial support.

Conflict of interest: The authors declare that there is no conflict of interest.

References

1. Castillo-Juarez , Gonzalez V, Jaime-Aguilar H, Martinez G, Linares E, Bye R, Romero I (2009). Anti-Helicobacter pylori activity of plants used in Mexican traditional medicine for gastrointestinal disorders. J Ethnopharmacol., 122: 402-40514. Liang R,
2. Cowan MM (1999). Plant products as antimicrobial agents. Clin. Microbiol. Rev., 12: 564-582.
3. Edris AE. Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents: a review. Phytother Res 2007; 21: 308-323.
4. Ga Ayoola H, Coker S, Adeg昀un A, Adepoju B, Obaweye E, Ezennia A (2008). Phytochemical screening and antioxidant activities of some selected medicinal plants used for Malaria therapy in Southwestern Nigeria. Trop. J. Pharmaceut. Res., 7(3): 1019-1024.
5. Gulluce M, Sahin F, Sokmen M, Ozer H, Daferara D, Sokmen A, Polissiou M, Adiguzel A, Ozkan H (2007). Antimicrobial and antioxidant properties of the essential oils and methanol extract from Mentha longifolia L. ssp. longifolia. Food Chem., 104(4): 1449-1456.
6. Hammons G.J., Fletcher J.V., Stepps K.R., Smith E.A., Baleentine D.A., Harbowy M.E. and Kadlubak F.F. (1999). Effects of chemoprotective agents on the metabolic activation of the carcinogenic alylamines PhIP and 4-aminobiphenyl in human and rat liver microsomes. Nutr. Cancer 33 pp.46–52.
7. Hideyuki I, Koji Y, Tae-Hoon K, Khennouf S, Gharzouli K, Yoshida T (2002). Dimeric and trimeric hydrolysable tannins from Quercus coccifera and Q. suber. J. Nat. Prod., 65: 339-345.
8. Horn R.C. and Vargas V.M.F. (2003). Antimutagenic activity of extracts of natural substances in the Salmonella/ microsome assay. Mutagenesis. 18 113-1.
9. Hussain AI, Anwar F, Nigam PS, Ashraf M, Gilani AH (2010). Seasonal variation in content, chemical composition and antimicrobial and cytotoxic activities of essential oils from four Mentha species. J Sci Food Agric.; 90: 1827-1836.
10. Ioannides C. and Lewis D.F. (2004). Cytochromes P450 in the bioactivation of chemicals. Curr. Top Med. Chem. 4 1767-1788.
11. Karakas V., Joshi S. and Shinde S.L. (2000). Antimutagenic profile of three antioxidant in the Ames assay and the Drosophila wing spot test. Mutat. Res., 468 183-194.
12. Khan IA, Abourashed EA (2010.). Leung's Encyclopedia of Common Natural Ingredients: Used in Food, Drugs and Cosmetics; 3rd ed.; John Wiley & Sons, Inc.: Hoboken, New Jersey.
13. Lopez V., Martin S, Gomez-Serranillos MP, Carretero ME, Jager AK, Calvo MI. Neuroprotective and neurochemical properties of mint extracts. Phytother Res 2010; 24: 869-874.
14. Lucchesi ME, Chemat F, Smadja J (2004). Solvent-free microwave extraction of essential oil from aromatic herbs: comparison with conventional hydrodistillation. J Chromatogr A; 1043: 323-327.
15. McKay DL, Blumberg JB (2006). A review of the bioactivity and potential health benefits of peppermint tea (Mentha piperita L.). Phytother Res; 20: 619-633.
16. Meng Z, Zhou Y, Lu J, Sugahara K, Xu S, Kodama H (2001). Effects of five flavanoid compounds isolated from Quercus dentata on superoxide generation in human neutrophils and phosphorelation of neutrophil proteins. Clinica Chimica Acta, 306: 97-102.
17. Patil T, Ishiuji Y, Yosipovitch G (2007). Menthol: A refreshing look at this compound. J. Am. Acad. Dermatol., 57: 873-878.
18. Rasosli I (2008). Dental Biofilm prevention by Mentha spicata and Eucalyptus camaldulensis essential oils. Int. J. Infect. Dis., 12(1): 167.
19. Sahgal G, Sreeramanan S, Sasidharan S, Xavier R, Ong MT (2009). Screening selected medicinal plants for antibacterial activity against Methicillin Resistant Staphylococcus aureus (MRSA). Adv. Nat. Appl. Sci., 3(3): 330-338.
20. Shimada K, Fujikawa K, Yahara K, Nakamura T (1992). Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. J Agric Food Chem ; 40: 945-948.
21. Stafford GI, Jager AK, van Staden J. Activity of traditional South African sedative and potentially CNS-acting plants in the GABA-benzodiazepine receptor assay. J Ethnopharmacol; 100: 210- 215.
22. Wadsworth TL, Koop DR (2001). Effects of Ginkgo biloba extract (EGb 761) and quercetin on lipopolysaccharide-induced release of nitric oxide.Chem Biot Interact; 137: 43-58.