

CHAPTER V

DISCUSSION

The *Salmonella* / reversion assay, constructed to monitor chemicals for mutagenicity / carcinogenicity (Ames, 1979), may also be applied for large-scale screening of natural and synthetic compounds for antimutagenic properties against various mutagens. Much of the evidence for the role of diet in cancer has been interpreted as evidence for the presence of carcinogenic factors in the diet (Lynnette, 1999). For example, the reactions between nitrite and dietary amines or amides under the acidic conditions of the stomach could lead to the formation of nitrosated products. It is possible that such products are the cause of the development of the gastric cancer in human (Mirvish, 1983). However, other chemicals may modulate carcinogenic and mutagenic activities. It is well known that ingredients in diet including edible plants, fruits and seeds may exert anticarcinogenic and antimutagenic activities (Hartman and Shankel, 1990; Hayatsu *et al.*, 1988). Realizing their importance, the present investigation has been conducted to evaluate the antimutagenicity of some herbal drinks.

Menstrual regulatory and haematinic traditional preparations, currently still popular among some Thai women, treated with excess of sodium nitrite in acid solution (pH 3.0-3.5) for 4 h showed their mutagenicity (แก้ว กังสดาลอำไพ และ วรณีย์ โรจนโพธิ์, 2531). Such reaction products were revealed their direct mutagenicity towards both

strains of *Salmonella typhimurium* (TA98 and TA100). This is an example of mutagens occurring during the simulating gastric digestion. Since the products of the model did not require metabolic activation before expressing their mutagenicity; it allowed the determination on the counteracting activity of any prospective antimutagen on mutation of stomach cells to be possible. Therefore, the effects of six herbal drinks on the mutagenicity of nitrite treated menstrual regulatory and haematinic traditional preparations were examined using the Ames test in the absence of metabolic activation.

5.1 Mutagenicity of Menstrual Regulatory and Haematinic Traditional Preparations

The finding obtained from this study demonstrated that none of the concentrate of menstrual regulatory and haematinic traditional preparations had any direct mutagens. The results from this study suggested that only the concentrated drugs treated with nitrite exerted the direct mutagenicity towards both strains of *Salmonella typhimurium* (TA98 and TA100) that could cause frame shift mutation and base-pair substitution. This is resemble to the study of Kangsadalampai and Rojanapo (แก้วกัสดาลอำไพ และ วรณี โรจนโพธิ์, 2531) who found that most menstrual regulatory and haematinic traditional preparations expressed their mutagenicity after being treated with nitrite on both *S. typhimurium* TA98 and TA100 without metabolic activation as well as the study of Sroysa-ard (1997) who revealed the mutagenicity of such drugs nitrosated

in vivo with nitrite in *Drosophila melanogaster* of the wing somatic mutation and recombination test.

Mutagenic effect of the nitrite treated menstrual regulatory and haematinic traditional preparations may be owing to some nitrosable components previously demonstrated to have mutagenicity after being interacted with nitrite in the acid condition. For example, pepper (*Piper nigrum* Linn, พริกไทย), nutmeg (*Myristica fragrans* Houtt, จันทน์เทศ), cinnamon (*Cinnamomum* sp., อบเชย), ceylon cinnamon (*Cinnamomum zeylenicum* Nees, อบเชยดังกา) contained some nitrite convertible precursors of direct mutagens (Namiki *et al*, 1984; Ungsurangsie *et al*, 1982). This is not surprised since nitrosating of extracts from plants e.g. Thai folklore medicinal herbs always generated some direct mutagens towards *S. typhimurium* TA98 and TA100 (Kangsadalampai *et al*, 1995). Therefore, consumers of any folklore medicines should avoid taking this type of drug before or after they have a chance to be exposed to nitrite containing food item.

5.2 Mutagenicity of herbal drinks

It is evident in this study that all extracts from herbs showed no mutagenicity on *Salmonella typhimurium* TA98 and TA100 in the absence of metabolic activating system. This information, at least, supports that some of the herbal drinks suggested by the Department of Health Promotion (Ministry of Public Health) to the consumer are safe. However, an unpublished observation on the nitrite treated extracts from these herbs in this laboratory showed their mutagenic potential that should be the precaution to the

health concerning consumers in simultaneously consuming such drinks with nitrite containing food items.

5.3 Effect of Herbal Drinks on Mutagenicity of Menstrual Regulatory and Haematinic Traditional Preparations

Although the main aim of this experiment was to reveal that the hot water extract from the studied herb were not only good herbal drinks but also had some beneficial as antimutagens. Unfortunately, most of them except those of *Hibiscus sabdariffa* Linn. and *Morus alba* Linn., showed their potentiating effect on the mutagenicity of nitrite treated menstrual regulatory and haematinic traditional preparations. Many of the chemicals described as antimutagens may also act as co-mutagens (Ferguson, 2001). Some constituents of the herbal drinks may increase the mutagenic efficiency with an unknown mechanism that was not specific to the mutagen. Tang and Edenharder (1997) reported that the juices from several fruits exerted weak to moderate co-mutagenic activity with respect to mutagenicity induced by nitroarenes in *Salmonella typhimurium* TA98. It was found that when some herbal extracts were incorporated into the pre-incubating tube composed of nitrite treated menstrual regulatory and haematinic traditional preparations and the tester strain, they increased the number of revertants of both *Salmonella typhimurium* strains TA98 and TA100 to be higher than that of the positive control tube.

Boiling of some herb generally releases most of the content in the plant cells to the water indicated by some pigments of the herbal drink; for instance, *Hibiscus*

sabdariffa Linn. (Roselle) drink shows its red color presented by some mixture of released pigments. Thus, some natural reductants, possibly be the constituent of the studied herbal extracts, may involved in the mutagenicity potentiation. Although there is no direct support for such hypothesis but it is an example for such consideration. Water-soluble reductants in the cytosol as well as fat-soluble reductants residing in cellular membranes were found in aerobic organisms (Dunlap *et al.*, 1997). Some biochemical reductant, namely NADH in the presence of Cu (II), could nonenzymatically convert a nitro-derivative portion of a toxicant such as heterocyclic amine (IQ) to be reactive species leading to oxidative DNA damage (Murata *et al.*, 1999). Therefore, a possible explanation on the increase of revertants induced by nitrite treated menstrual regulatory and haematinic traditional preparations may be the effect of some reductants released from herbs during hot water extraction.

The induction of herbs on enzymes, namely, nitroreductase and O-acetyltransferase in *Salmonella typhimurium* of Ames test that are believed to play a role in mutagenesis of positive standard should be investigated. In addition, the antimutagenicity of such herbal drinks should be performed on other testing systems will warrant the effect of the herbs found in the present experiment. Finally, difference between bacterial cells (*in vitro*) and mammalian tissues (*in vivo*) may be a limit in interpretation of such benefit of herbal drinks; therefore, examination for their antimutagenic activity in animal model are recommended.

5.4 Consumer Interpretation

This study suggests that the non-enzymatic formation of direct-acting mutagens such as products from treating menstrual regulatory and haematinic traditional preparations with nitrite in acidic solution (pH 3.0-3.5) may be concerning physiologically important cause regarding to the etiology of gastric cancer. Much attention has been focused on the antimutagens probably presented in herbal drinks commonly consumed by Thai people. However, it was shown that samples of the present study potentiated number of revertants induced by nitrite treated menstrual regulatory and haematinic traditional preparations. It was revealed that herbal drinks not only posed some health benefit but also increased the risk of consumers who consumed some toxicants especially the ones produced during nitrosation. Thus, avoiding consumption of nitrite containing foods should be a better way to such risk.