PHENOLIC COMPOUNDS INTERFERE IN THE ESTIMATION OF COPPER BY DIETHYLDITHIOCARBAMATE METHOD

A. P. S. NARANG and R. L. MATtoo*
Department of Clinical Biochemistry
Sher-I-Kashmir Institute of Medical Sciences
SRINagar-190 011, J & K.

Received on October 7, 1989; Revised on September 17, 1990.

ABSTRACT

For the estimation of copper, diethyldithiocarbamate method is most commonly employed in various laboratories where atomic absorption spectrophotometer is not available. The prevalence rate of gastrointestinal malignancies is very high in Kashmir as compared to other parts of the world. We could find high serum copper levels in gastrointestinal tract cancer. Intake of salted tea, prepared in copper vessel, is very common in Kashmir. Such tea samples showed copper value as 3168.9 (S.D. 700.5) µg/dl estimated by diethyldithiocarbamate method. When these samples analysed by atomic absorption spectrophotometer, the copper levels were just 9% as reported by colorimetric method. Various black salted tea samples were prepared in the laboratory in glass vessel. The mean copper was 1115.0 (S.D. 350.4) µg/dl. After addition of milk, the values were reduced by 50%. Nine phenolic compounds showed varying amount of copper by colorimetric method and no copper could be detected by atomic absorption spectrophotometer. Phenolic compounds present in tea leaves interfere in the estimation of copper by diethyldithiocarbamate method. It is suggested that diethyldithiocarbamate colorimetric method for copper estimation is not suitable for solutions containing phenolic compounds.

INTRODUCTION

Diethyldithiocarbamate method (1) is widely used for the colorimetric estimation of copper in serum, urine and other samples in such laboratories which lack atomic absorption spectrophotometric facilities. In this method copper, after being dissociated from proteins by hydrochloric acid and deproteinisation with trichloroacetic acid, is extracted in a mixture of amyl alcohol and ether and then complexed with sodium diethyldithiocarbamate as a golden yellow coloured compound which is directly measured at 440 nm.

Earlier reports from this laboratory (2–4) revealed an increase in serum copper in cancer patients in general with a greater increase in gastrointestinal tract cancer patients.
in particular. Since the occurrence of gastrointestinal tract cancer in Kashmiri population is high, a number of dietary constituents especially consumed in this part of the world, are suspected to possess carcinogens or their precursors (5). One such dietary habit includes consumption of hot pink-coloured salted tea, on an average of five cups per day, which is prepared in copper vessel known as “Samawar”. Special type of green tea leaves are brewed in the presence of sodium bicarbonate for 2–4 hours with intermittent replenishment of water. Then salt and milk are added during the final boil prior to consumption. In order to assess any relation between dietary copper intake and serum copper, salted tea samples were chosen as one of the environmental factors.

Such tea samples brought by the relatives of patients when analysed for copper by colorimetric method (Diethyldithiocarbamate method) showed variable but high amounts of copper. Subsequently, tea samples were analysed on an atomic absorption spectrophotometer which revealed nearly one tenth of the amount of copper as estimated by colorimetric method. Since tea leaves are known to contain phenolic compounds which are readily extractable under alkaline conditions, such as sodium bicarbonate, it was interesting to examine the extent of interference, if any, by phenolic compounds in the estimation of copper by diethyldithiocarbamate method.

MATERIAL AND METHODS

Experiments were conducted to brew forty tea preparations in the laboratory in glass vessels under controlled conditions; 2 g of tea leaves with 200 mg of sodium bicarbonate were boiled with water for three hours and then 200 mg of sodium chloride was added and the final volume of the brew made to 200 ml. To ten such brews, 25 ml of cow’s milk was added per brew, while 25 ml of water was added to other thirty (black) extracts and analyses for copper carried out colorimetrically (1) and by atomic absorption spectrophotometrically (Pye-Unicam SP-2900). The assay by the latter method involved mixing of sample with equal volume of cold 25% trichloroacetic acid, chilling in ice bath for 30 minutes followed by centrifugation and the supernatant was directly aspirated into atomic absorption spectrophotometer.

Nine phenolic compounds of analytical grade were obtained and their solutions were prepared (20 mg/dl each). Copper was measured using diethyldithiocarbamate method and by atomic absorption spectrophotometer.

RESULTS

The results of copper assayed by two methods in tea samples, prepared by conventional methods in Samawar and in the laboratory, are represented in Table 1. Copper in black tea estimated by colorimetric method was found to be in the range of 386 to 1662 with the mean of 1115.0 (S.D. 350.4) µg/dl and was nearly 40 times than those determined by atomic absorption spectrophotometer. After addition of milk, the colorimetrically determined values for copper were reduced by 50%, while a 50% increase was recorded in the values assayed by atomic absorption spectrophotometer, which was expected because