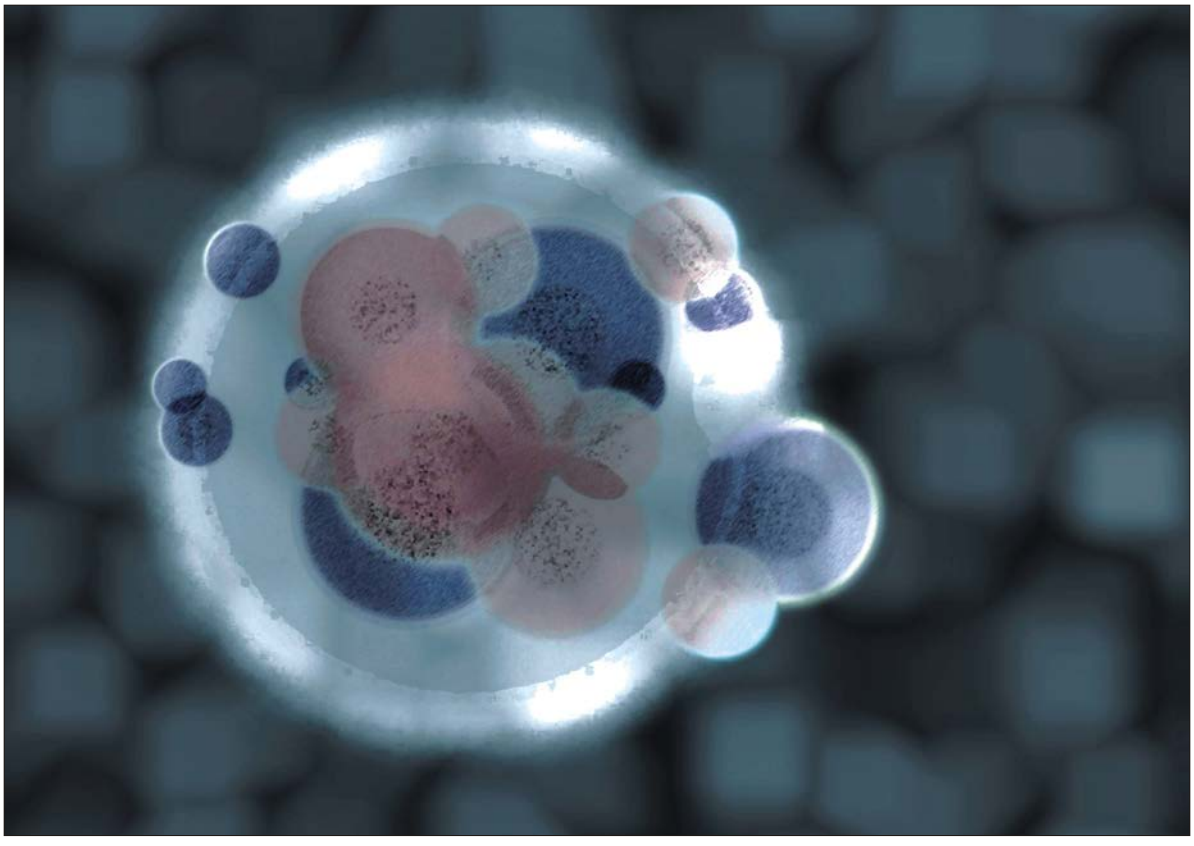

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Medicinal Use of *Camellia sinensis* on Lactose Intolerance

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Abstract: The present investigation was aimed at to test different *Camellia sinensis* (tea) extracts such as Magholai (I), 3 roses (II) and chakra gold (III) for their lactose reduction capability in milk with more lactose. Cow, goat, buffalo and two commercial milk samples such as KC and Star were tested for its lactose content. Among these buffalo milk as used as a control due to its high content of lactose (8 mL dL^{-1}). Buffalo milk was treated by tea extracts I, II and III and 0.018, 0.018 and 0.042 mg dL^{-1} of lactose content were found. Its reveals that the lactose content of milk was well reduced by adding tea extracts and also its suggested peoples to have milk with herbal extracts (tea) who have gastrointestinal problems.

Key words: Lactose intolerance, milk samples, *Camellia sinensis,* gastrointestinal problem

INTRODUCTION

Green tea is popular in Asia, including Japan, although it is rarely consumed by Western people. It contains large amounts of catechins which are potent free radical scavengers and therefore, has an antioxidant effect (Nagaya *et al*., 2004; Wiseman *et al*., 1996). Oxidative stress participates in the pathogenesis of many cardiovascular diseases. Recently, black tea consumption has been shown to reverse endothelial dysfunction in patients with coronary artery disease through its antioxidant effects (Duffy *et al*., 2001). However, the number of catechins in non-fermented tea (green tea) is higher than that in fermented tea (black tea) (Wiseman *et al*., 1996). These findings raise the possibility that green tea acts as a potent antioxidant and medicinal plant (Nagaya *et al*., 2004).

Significant changes in our knowledge and approach towards, lactose intolerance have occurred over the past quarter century, since the first statement on lactose intolerance was published by the American Academy of Pediatrics Committee on Nutrition in 1987. The enzyme lactase phlorizin hydrolase, located at the intestinal brush border, is necessary for the hydrolysis of lactose, the main sugar in milk. Due to the genetically programmed decrease in intestinal lactase activity that occurs post-weaning (lactase non-persistence) (Khabarova *et al*., 2009) a large proportion of the human population loses, in adult age, the possibility to digest and absorb lactose (Argnani *et al*., 2008). Symptoms of excessive gas, stomach distention and even diarrhea may occur if excessive lactose is consumed. According to the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), up to 75% of all adult African Americans and Native Americans and 90% of Asian Americans have the potential for symptoms of lactose intolerance. In Europe, its prevalence increases with a North-South and West-East gradient. Thus, about 50% of adult Italians cannot digest and absorb lactose normally (Argnani *et al*., 2008; Burgio *et al*., 1984; Bozzani *et al*., 1986). Accordingly, the purpose of this study was to investigate whether milk with and without *Camellia sinensis* extracts I, II and III consumption reduce the lactose content. Cow, goat, buffalo and two commercially available milks (KC and Star) were used for lactose content determination.

MATERIALS AND METHODS

This research work was conducted from December 2007 to March 2008. All the chemicals used in this study were supplied by Sigma (USA) and Genei (Bangalore). The 2.5 g of lactose monohydrate (USP grade) was dissolved in 200 mL of 0.1% Benzoic acid and stored in refrigeration. 10, 15, 20, 25 and 30 mL of stock solution was diluted to 250 mL separately to get 0.5 to 1.5 mg lactose mL^{-1}. Then 40 g of NaOH was dissolved in

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1 L of CO_2 free water. Zinc Acetate-Phosphotungstic acid (ZAPT) reagents was prepared by using 3 g of zinc acetate and 1 g of phosphotungstic acid were dissolved in water. The 2.5 mL of glacial acetic acid was added and made upto 25 mL using distilled water. Glycine-NaOH buffer was prepared by using 510 mL of glycine solution containing 0.247 g of glycine dissolved in 15 mL distilled water and 1 g of NaCl, 340 mL of 1 N NaOH were mixed to give a pH 12.8. For preparing Methylamine solution 0.5 g of methylamine was dissolved in 10 mL distilled water and stored in refrigerator. For Sodium sulphate 1% (w/v) solution, 1.0 g was dissolved in distilled water and diluted to 100 mL.

Milk and tea leaves samples: Cow, goat, buffalo milks and commercial brands such as KC and Star milks were used to determine the presence of lactose content. Magholai tea (I), 3 roses (II) and Chakra gold tea (III) extracts 8.0 mL were individually dissolved in distilled water. The determination of lactose in various milk sources was done by using spectrophotometer. This method was based upon the reaction of lactose with methylamine in hot alkaline solution to form a red complex which absorbs measured spectrometrically at 540 nm.

Lactose content of milk and impact of extracts in milk samples were determined by the following formula:

$$\text{Lactose content in milk/milk with extracts} = \frac{\text{TestOD}}{\text{StdOD}} \times \text{Conc. of Std} \times \frac{1}{\text{DF}}$$

where, OD is optical density, DF is dilution factor and Std is standard.

Herbal extracts used in this experiment were weighed as 10 g of each and dissolved in 50 mL of distilled water and filtered. Fifteen milliliter of filtrate was added to 35 mL of milk; about this 8.0 mL was used for test. The 8.0 mL of well missed samples, 1.0 mL of ZAPT reagent was added and diluted to 10 mL and incubated for 10 min and filtered the contents using Whatman No.1 filter paper.

Working standard for lactose in milk was processed of each from constant volume 5.0 mL and the working standard concentration ranges from 0.5-1.5 mg dL^{-1}. With 0.5 mL of filtrate, 0.5 mL of NaOH solution was added and diluted to 10 mL. Then the 5.0 mL of filtrate was diluted to 10 mL from that take 5.0 mL of filtrate was treated with remaining reagents such as buffer (5.0 mL), methylamine (0.5 mL) and sodium sulphate (0.5 mL). Five milliliter of each of working standard lactose and unknown solution was pipette out into 15 mL test tubes. Five milliliter of glycine NaOH buffer, 0.5 mL of sodium sulphite solution was added in each tube and mixed thoroughly. Tubes were heated in a thermostatically controlled water bath at 65°C for 25 min and cooled immediately in an ice water bath for 2 min to stop the reaction. Absorbance against blank at 540 nm was recorded in a spectrophotometer. A standard curve is drawn by plotting absorbance against concentration of lactose and lactose level was determined.

RESULTS

Working standard for lactose in milk was processed of each from different concentration ranges from 0.5-1.5 mg dL^{-1}. The various standard samples were processed with the reagents such as buffer (5.0 mL), methylamine (0.5 mL) and sodium sulphate (0.5 mL). The resultant colored sample was measured spectrophotometrically at 540 nm. Figure 2 shows the OD values of standard lactose were 0.042, 0.102, 0.179, 0.245 and 0.311 mg dL^{-1} in respect with the concentration of 0.5, 0.75, 1.00, 1.25 and 1.50 mg (Fig. 1).

Five milk samples were tested for its lactose content. The lactose content were in cow milk 3.97 mg dL^{-1}, goat milk 3.89 mg dL^{-1}, buffalo milk 8.24 mg dL^{-1}, KC milk 5.83 mg dL^{-1} and star milk 3.40 mg dL^{-1} were observed (Fig. 2). The star milk was shows slight decrease in lactose

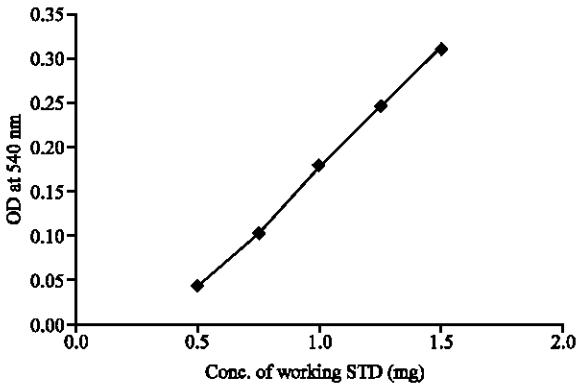

Fig. 1: Different concentration of known strength of lactose in milk (mg)

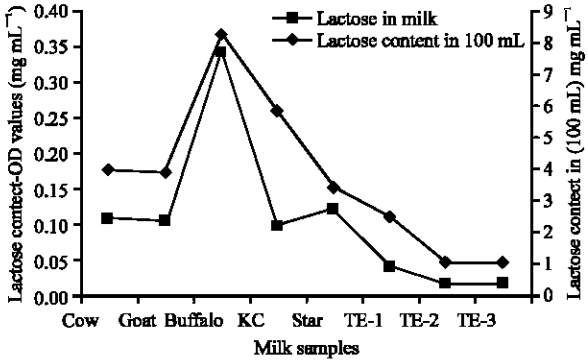

Fig. 2: The amount of lactose content in the milk samples (mg mL^{-1})

content. This may be the centrifugation or some other processing remove the amount of lactose from the natural milk. Among these, buffalo milk was used as a control due to it high content of lactose (8.24 mg dL^{-1}).

Thirty five milliliter of control sample with 15 mL of tea extract I were processed by the prescribed methods, from this 5 mL of filtrate was treated with remaining reagents. The same procedure was applied for rest of the tea extracts like II and III. Figure 2 showed the results of lactose content reduction in control milk sample with different extracts like I-2.50, II-1.07 and III-1.07 mg dL^{-1}. The variation between control (buffalo) and extracts with control (tea + buffalo milk) were I-5.74, II-7.17 and III-7.17 mg dL^{-1}. Among these tea extracts II and III shared the common value (7.17 mg dL^{-1}) which was found to be more active towards the reduction of lactose content of buffalo milk from initial level of 8.24 to 1.07 mg dL^{-1} and followed by tea extracts I (8.24 to 2.50 mg dL^{-1}). It reveals the lactose content of buffalo milk was well reduced by adding tea extracts. Moreover, it is preferably good to acquire the milk with tea extracts rather than milk alone, especially for lactose intolerant patients.

DISCUSSION

Pharmaceutical preparations of fungal or yeast derived β-galactosidase have been developed for the treatment of lactose maldigestion. There is evidence that these preparations increase lactose digestion and alleviate symptoms (Moskovitz *et al*., 1987; Sanders *et al*., 1992) but different preparations seem to vary in their effectiveness (Ramirez *et al*., 1994) and they do not help all subjects (Moskovitz *et al*., 1987). Compared to lactose in yoghurt or in pre-hydrolyzed milk, these products seem less efficient (Onwulata *et al*., 1989). Seven epidemiological studies (one cohort and six casecontrol studies) with a total number of 77,777 subjects showed inverse association of green tea consumption (urine polyphenol epigallocatechin in one study) and the risk reduction of stomach cancer (Liu *et al*., 2008; Sun *et al*., 2002; Mu *et al*., 2003; Sasazuki *et al*., 2004; Liu *et al*., 2008; Yu *et al*., 1995) The cohort study with 72,943 subjects showed benefit for women who consumed five or more cups of green tea per day (RR 0.51; 95% CI, 0.30-0.86) compared with one cup per day (Sasazuki *et al*., 2004). However, four studies including two cohort studies with 102,179 subjects did not show an inverse association of green tea consumption and risk reduction of stomach cancer or cancer-caused death (Liu *et al*., 2008; Hoshiyama *et al*., 2002, 2004; Sasazuki *et al*., 2004).

A randomized trial comparing green tea, black tea with water in 143 heavy smokers found significant decrease in 8-OHdG levels after a 4-month intervention (Hakim *et al*., 2003). A prospective cohort study in 52 male smokers demonstrated that drinking green tea inhibited cigarette-induced increase in sister chromatid exchange rates (Shim *et al*., 1995). A prospective cohort study followed 20,550 men and 29,671 women for an average of 10.3 years and estimated the HRs (95% CI) in oral cancer (Ide *et al*., 2007). For women, the HRs (95% CI) were 0.51 (0.10-2.68), 0.60 (0.17-2.10) and 0.31 (0.09-1.07) for green tea consumption of one to two, three to four and five or more cups per day, respectively, compared with those who drank less than one cup per day (P for trend, 0.08). For men, no trend was observed. However, no severe adverse effects have been reported in association with the medicinal use of green tea. Consumption of high doses of green tea or green tea extract (i.e., 5-6 L per day) may cause nausea, vomiting, abdominal bloating/pain, dyspepsia, flatulence and diarrhea. Excessive consumption of caffeine from green tea may also cause central nervous system stimulation such as dizziness, insomnia, tremors, restlessness, confusion, diuresis (i.e., increasing urine output), heart rate irregularities and psychomotor agitation (Laurie *et al*., 2005).

Argnani *et al*. (2008) reported that oral load of 12.5 g of lactose, corresponding to about 250 mL milk, is well tolerated by the majority of patients unable to completely digest and absorb 25 g lactose. The present result confirm high amount of lactose in buffalo milk (82.4 mg corresponding to 1 L of milk) (Fig. 2). It is suggest that 12.5 g lactose (250 mL) content milk should be preferred to consume while milk with more lactose. The present investigation showed that 250 mL buffalo milk was contains 20.6 mg lactose; it was more than other milk samples such as cow milk 9.93 mg, goat milk 9.73 mg, KC milk 14.55 mg and Star milk 8.5 mg and the observation (12.5 g) of Argnani *et al*. (2008). It suggests that consumption of this buffalo milk with more lactose may be inducing abdominal pain and diarrhea in most lactose malabsorbers.

In the present study, reduction of lactose content in control buffalo milk by tea extracts I, II and III were used. Among these tea extracts II and III shared the common value (1.07 mg dL^{-1}) which was found to be more active towards the reduction of lactose content of buffalo milk from initial level of 8.24 to 1.07 mg dL^{-1} (8.24-1.07 = 7.17 mg dL^{-1}) and followed by tea extracts I from 8.24 to 2.50 mg dL^{-1} (8.24-2.50 = 5.74 mg dL^{-1}). Extracts II and III which were found to be more active towards the reduction of lactose content (7.17 mg dL^{-1}) of

buffalo milk; it is reveals the lactose content of buffalo milk was well reduced by adding tea extracts II and III. In addition, it is suggest that 2.68 mg lactose presence in 250 mL control milk when it was treated by extract II and III while extract I shows 6.25 mg lactose in 250 mL control milk. The present investigation showed that 250 mL control milk (buffalo) sample was contains 20.6 mg lactose and after the treatment by extracts I, II and III it was reduced the lactose content from 20.6 mg to 6.25, 2.68 and 2.68 mg which reveals that consumption of this milk with tea extracts could be reducing the lactose content and also its suggested peoples to have milk with herbal extracts (tea) who have gastrointestinal problems inducing abdominal pain and diarrhea in most lactose malabsorbers. Before suggesting our results, no detailed investigation was made on treating lactose intolerance by tea extracts.

CONCLUSION

As tea drinking is common in many populations; thereby they are alleviating the gastro intestinal problems due to lactose intolerance without knowing scientific background. Now from our observation that is proved that tea extracts along with milk reduce the content of lactose. Thus, green tea consumption may be beneficial for the prevention and treatment of lactose intolerance. Further prospective, randomized studies of green tea consumption are necessary to examine whether green tea consumption reduces the risk of gastro intestinal problems such as bloating, flatulence, diarrhea, gas production and stomach cramp events and mortality.

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