Determination of Trace Elements in Cow's Milk in Pathumtani Province

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Abstract

Milk and dairy products make an important contribution to the supply of nutrients for the human diet, since it is a source of some vitamins and a lot of mineral constituents which are necessary for proper development and functioning of different tissues and organs. This study was directed to measure the concentrations of Zinc (Zn), Cadmium (Cd), Copper (Cu) and Lead (Pb) in cow's milk samples available commercially in Pathumtani Province using Inductively Coupled Plasma-Optical Emission Spectrometer.

The aim of the present study is to determine the concentrations of some trace elements, namely Zinc (Zn), Cadmium (Cd), Copper (Cu) and Lead (Pb) in cow's milk available in markets of Pathumtani Province. Two types of milk samples were analyzed including fresh cow's milk and liquid milk prepared from cow's milk powder. The mean elemental concentration values in ppb of Cr, Cu, Zn, Cd, and Pb in fresh cow's milk were: (715.1 ± 7.1), (36.8 ± 1.8), (2.4 ± 0.3) and (1.2 ± 0.1) respectively. Those in cow's milk powder were: (20.6 ± 0.8), (36.9 ± 1.1), (956.8 ± 3.2), (3.1 ± 0.3), and (2.2 ± 0.2) respectively.

Keywords: Inductively Coupled Plasma-Optical Emission Spectrometry; cadmium; copper; lead; zinc; cow's milk

Introduction

Elements are essential micro-nutrients and have a variety of biochemical functions in all living organisms. Some of them form an integral part of several enzymes. Although they are essential, they can be toxic when taken in excess; both toxicity and necessity vary from element to element and from species to species. Thus, information

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on the intake of heavy metals through food chain is important in assessing risk to human health.

The world-wide contamination of milk with undesirable substances via animal feeds, heavy metals, mycotoxins, dioxins and similar pollutants is considered to be of great concern to public health due to their toxic effects on humans and wildlife. Recent report according to Semaghiul *et al*. indicated that good quality measurements are essential to control and often play a vital role in maintaining products and process quality in manufacturing, trade and research. In recent years, there has been a growing interest in microelements as their presence in foods is the indicator of qualitative parameters such as processing conditions, environmental pollution, sanitation and husbandry and may affect the chemical and functional properties of milk.

Milk and dairy products make an important contribution to the supply of nutrients for the human diet. The ash of cow's milk contains some major elements such as calcium, phosphorus and magnesium, in addition to potassium, sodium and chlorine and a wide range of trace elements including zinc, copper, iron, manganese and iodine. Thus cow’s milk is an important source of protein, minerals and vitamins in the human diet. However, contamination of milk and dairy products by toxic metals can be a possible health risk to human population. The presence of toxic metals in the food chain is the result of environmental pollution and their concentrations need to be controlled constantly. The composition of the mineral fraction of milk and milk products has been frequently considered, but only a few published investigations deal with minor but only a constituent in food..

The aim of the present study is to determine the concentrations of some trace elements, namely Zinc (Zn), Cadmium (Cd), Copper (Cu) and Lead (Pb) in cow's milk available in markets of Pathumtani Province. The studied milk samples were fresh cow's milk and liquid milk prepared from cow’s milk powder available in Pathumtani Province market. A total of 55 samples were analyzed after “Wet digestion" for five trace elements using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES). The daily

intakes of these elements are also compared with the Recommended Dietary Allowance (RDA) values set by different international Organizations.

Aim of Research

The aim of the present study is to determine the concentrations of some trace elements, namely Zinc (Zn), Cadmium (Cd), Copper (Cu) and Lead (Pb) in cow's milk available in markets of Pathumtani Province.

Experimental Procedure

Collection of milk samples

For the present study, the commercially available fresh cow's milk samples and liquid milk prepared from cow's milk powder were collected from different supermarkets in Pathumtani Province.

Reagents

Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) standard solutions for Zn, Cd, Cu, and Pb were purchased from Fisher Scientific Company, USA. Working standard solutions were prepared by diluting the stock solution. Sulfuric acid, perchloric acid and nitric acid were all of AR quality. De-ionized water has been used when required.

Sample Digestion and Preparation of Analyte Solution for ICP-OES

The milk sample needs to be brought into clear solution for analysis by the Inductively Coupled Plasma-Optical Emission Spectrometer. For this reason the milk sample was first digested with chemicals where the organic matrix of milk was destroyed and the elements is left into a clear solution. "Wet Digestion" method (i.e. digestion with nitric, sulfuric and perchloric acids) has been used in the present study.

Calibration Curve

The calibration curves were drawn for Zn, Cd, Cu, and Pb by Macintosh Microcomputer using linear regression analysis of the concentrations of the standard solutions versus absorbance values. A new calibration curve was plotted for each

element every time a new batch of milk samples was arranged for analysis. Each standard solution was measured at least three times and the mean was plotted.

Measurement of elemental concentration in milk samples

10 µl aliquot of milk samples, obtained after wet digestion, was injected into the ICP-OES with the help of an auto-sampler, and the elemental concentration was read from the output of the printer connected to the computer. Each sample was repeated three times for each element. The concentrations of Zn, Cd, Cu and Pb were determined for each sample. A total of 55 milk samples were analyzed in the present study.

Results and Discussions

The range of linearity of concentration vs. absorbance curve is of great importance in determining the elemental concentration of the milk samples. The calibration curves for Zinc (Zn) and Cadmium (Cd) are shown in Figure 1 and Copper (Cu) and Lead (Pb) are shown in Figure 2

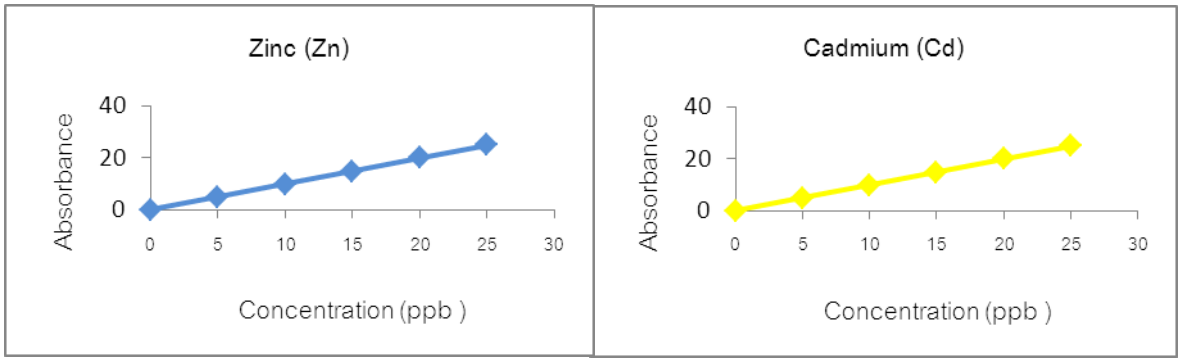

Figure 1 Concentration versus absorbance curves for Zinc (Zn) and Cadmium (Cd)

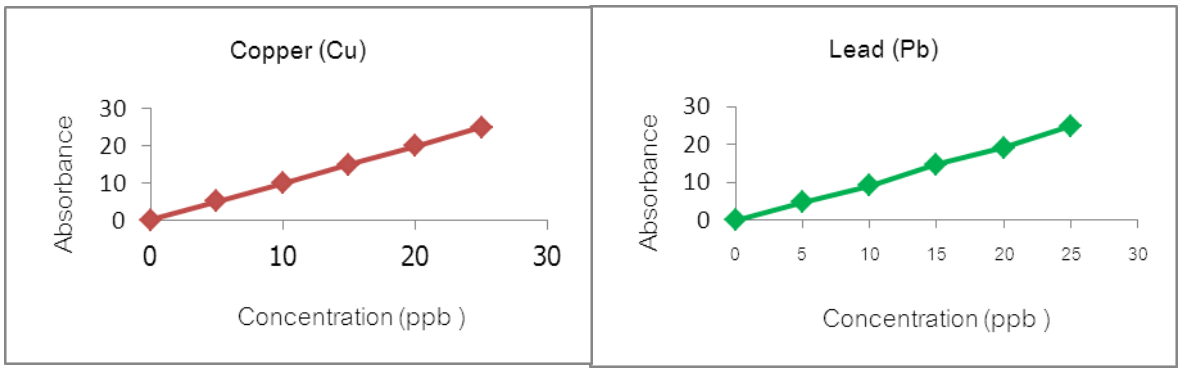

Figure 2 Concentration versus absorbance curves for Copper (Cu) and Lead (Pb)

The results of the present study for four trace elements in milk samples collected from different areas in Pathumtani Province are given in Tables 1 and 2. The elemental concentrations in fresh cow's milk samples are presented in Table 1, while the concentrations in liquid milk prepared from cow's milk powder are given in Table 2.

Table 1 Elemental concentration in fresh cow's milk in Pathumtani Province

Type of Milk	Brand Name	Concentration (µg/kg)			
		Zinc	Cadmium	Copper	Lead
Fresh Cow's Milk	A	599.2 ± 6.7	1.0 ± 0.0	51.1 ± 2.0	1.1± 0.1
	B	352.3 ± 5.2	2.0 ± 0.2	53.0 ± 1.9	1.1 ± 0.2
	C	1115.1 ± 8.6	4.1 ± 0.5	53.1 ± 2.1	3.2 ± 0.4
	D	1144.3 ± 8.9	2.0 ± 0.4	43.1± 1.7	1.0 ± 0.0
	E	1180.0 ± 9.2	5.1 ± 0.6	27.2 ± 1.	1.0 ± 0.1
	F	990.2 ± 7.3	5.1 ± 0.8	41.0 ± 1.6	5.2 ± 1.0
Mean		896.8 ± 7.6	3.2 ± 0.4	44.7 ± 1.7	2.1 ± 0.3

In fresh cow's milk, the mean concentration of Zn is the highest (896.8 ± 7.6)g/kg followed by Cu (44.7 ± 1.73) µg/kg, Cd (3.2 ± 0.4) µg/kg and the last Pb (2.1 ± 0.3) µg/kg.

Table 2 Elemental concentration in liquid milk prepared from cow's milk powder in Pathumtani Province

Type of Milk	Brand Name	Concentration (µg/kg)			
		Zinc	Cadmium	Copper	Lead
Liquid milk prepared from cow's milk powder	a	623.2 ± 5.8	1.0 ± 0.0	35.0 ± 2.2	1.1 ± 0.1
	b	352.3 ± 5.2	2.0 ± 0.2	53.0 ± 1.9	1.1 ± 0.2
	c	908.4 ± 6.1	1.1 ± 0.1	24.2 ± 2.0	1.1 ± 0.1
	d	1124.3 ± 8.9	2.0 ± 0.4	43.1 ± 1.7	1.0 ± 0.0
	e	200.4 ± 9.3	2.2 ± 0.6	27.2 ± 1.9	1.0 ± 0.1
	f	990.2 ± 7.3	5.1 ± 0.8	36.9 ± 1.1	2.2 ± 0.2
Mean		715.1 ± 7.1	2.4 ± 0.3	36.8 ± 1.8	1.2 ± 0.1

In liquid milk prepared from cow's milk powder, the mean concentration of Zn is the highest (715.1 ± 7.1) µg/kg followed by Cu (36.8 ± 1.8) µg/kg, Cd (2.4 ± 0.3) µg/kg and the last Pb (1.2 ± 0.1) µg/kg.

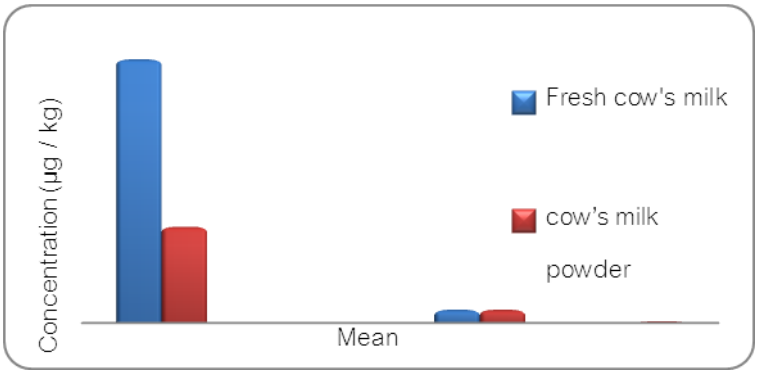

Figure 3 Distribution of concentration of four trace elements in fresh cow's milk and in Liquid milk prepared from cow's milk powder

The distribution of concentrations of four trace elements in both milk samples are shown in Figure 3. The elemental concentrations in fresh cow's milk are higher than the corresponding values in liquid milk prepared from cow's milk powder except for Zn concentration. The Zn concentration is higher in liquid milk prepared from milk powder than in fresh milk.

Table 3 Comparison of the elemental concentrations of fresh cow's milk in Pathumtani Province with the published values

Country	Concentration (µg/kg)			
	Zinc	Cadmium	Copper	Lead
Japan	3000.0	1.0	100.0	50.0
Germany	3730.0	0.1	49.9	5.5
India	3100.0	0.1	56.0	1.6
Spain	1419.3	----	9.7	49.3
Poland	3770.0	15.0	90.0	20.0
USA	2235.2	9.7	19.4	34.0
Pathumtani Province	896.8	3.2	44.7	2.1

There are wide variations in the published data for the elemental concentrations of cow's milk of different countries. Some of the results are recorded in Table 3 for comparison with the present values. The Cu concentrations of the present study are well comparable with data. But the present value of Zn concentration is the lowest compared with the corresponding values of other countries. This shows that the cow's milk in Saudi Arabia is a poor source of Zinc. The cow's milk in Pathumtani Province is also a poor source of Cd and Pb.

The daily intake of the metals depends on both the concentration and the amount of food consumed. The reported values of daily milk consumption in USA and Spain are respectively 224 g and 124 g

Table 4 Comparison of daily intakes of metals from 124 g of milk by Pathumtani Province population with the recommended values from all food intake.

Element al Concentration (µg/day)	Fresh cow's milk	Cow's milk powder	Recommended / Permissible value (µg/day)
Copper	5.9	4.4	2000 - 3000
Zinc	113.9	115.3	12000 - 15000
Cadmium	0.6	0.4	57 - 72
Lead	0.4	0.3	429

Table 5 Percentage of recommended/permissible dietary allowances per 124 g of Cow's milk.

Element all	USA	Spain	Pathumtani Province
Copper	0.3	0.1	0.3
Zinc	2.3	1.5	2.3
Cadmium	2.1	-	2.1
Lead	0.9	1.4	0.9

Assuming a value of 124 g / day for milk consumption in Pathumtani Province, the daily intake of these metals are determined and are depicted in Table 4. The last column shows the Recommended Dietary Allowance (RDA) as set by different international organizations Prepared from milk powder than in fresh milk. The last column shows the Recommended Dietary Allowance (RDA) as set by different international organizations.

Percentages of Recommended Dietary Allowance per 124 g of cow's milk for different countries are presented in Table 5. The present values of Cu of cow's milk are well comparable with the values for some other countries. But the cow's milk in Pathumtani Province are poor sources of Zn, Cd, and Pb supplying less than 1% of the estimated RDA in a 124 g serving.

Conclusion

In fresh cow's milk and liquid milk prepared from cow's milk powder. The result mean elemental concentrations in ppb of Cu, Zn, Cd, and Pb in fresh cow's milk were that the concentration of Zn is the highest followed by Cu, Cd and Pb. The elemental concentrations in fresh cow's milk are higher than those in liquid milk prepared from cow's milk powder except for Zn of which concentration is higher in liquid milk prepared from milk powder than in fresh milk.

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Bioactivity of Gynura divaricata DC. Ethanol crude extract

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Abstract

The aim of this research was to study bioactivity of the ethanol crude extract of *Gynura divaricata* DC. In reducing in blood sugar level in diabetes rats. Fresh *Gynura divaricata* DC.leaves were macerated with ethanol. 200–350 g. of Spraque-Dawley' s rats, both male and female, were randomly sampled.The rats were divided into 3 control and 3 experimental groups, with 9 rats for each group. The experimental groups were orally daily fed with 2.90 mg/kg.bw. 5.79 mg/kg.bw. And 11.43 mg/kg.bw. Of crude extract for 4 weeks whereas the control were fed with water and control solvent. The diabetes rats receiving 2.90 mg/kg.bw. Exhibited the lowering of blood sugar for 4 weeks after receiving crude extract. The blood sugar level was not significantly different compared with the normal control group and compare control group on the first and third weeks, but significantly increased on the second and forth weeks ($p<0.05$). Both 5.79 mg/kg.bw. And 11.43 mg/kg.bw. groups were shown to have the high blood sugar level with the significance different comparing with the normal control group and compare control group ($p<0.05$) for 4 weeks after receiving crude extract.

Keywords: Gynura divaricata DC., ethanol crude extract, bioactivity

Introduction

Gynura divaricata DC. Is a medicinal plant, traditionally used to treat diabetes mellitus, cancer, hypertension, and hyperlipidemia as well as for health promotion. The bioactivity of Gynura divaricata DC. Ethanol crude extract to reduced blood sugar level have not been studied by scientific process. Thus, this research aimed to study crude extract of *Gynura divaricata* DC. in reducing in blood sugar in diabetes rats.

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Materials and Methods

Plant materials

The fresh leaves of Gynura divaricata DC.Were collected from Singburi province.

Preparation of extract

The fresh leaves were pulverized into powder. The powder was extracted with ethanol by maceration and evaporated with the vacuum rotary evaporator, dried-viscous dark green extract was obtained and the yield was 0.48%.

Animals

The animals were purchased from National Animal Research center, Salaya, Thailand. They were in standard polyethylene cage at Animal Husbandry and Research Unit, Faculty of Allied Health Sciences, Burapha University, Chonburi, Thailand. Animals are acclimatized for two weeks in 24-25 degree Celcius in 60 percent humidity room under 12:12 hr.light dark ratio.

Methods

200–350 g. of Spraque-Dawley's rats, both male and female, were randomly sampled.The rats were divided into 3 control groups (normal, compare and diabetes control groups) and 3 experimental groups with 9 rats for each group. The experimental groups were orally daily fed with 2.90 mg/kg.bw. 5.79 mg/kg.bw. And 11.43mg/kg.bw. of crude extract for 4 weeks. The control groups were fed with water and control solvent. The diabetes control group was injected with Streptozotocin. The blood sugar of rats of control and experimental groups were checked for 4 weeks.

Results and Discussion

The results of the reduction of blood sugar level in diabetes rats are shown in Table 1

Table 1 blood sugar level in diabetes rats

groups	Blood sugar level (mg/dl) (before the experiment)	Blood sugar level(mg/dl) (after receiving Streptozotocin) (1st week)	Blood sugar level(mg/dl) (after receiving crude extract) (1st week)	Blood sugar level(mg/dl) (after receiving crude extract) (2nd week)	Blood sugar level(mg/dl) (after receiving crude extract) (3rd week)	Blood sugar level(mg/dl) (after receiving crude extract) (4th week)
control gr. (normal)	83.1±9.6	93.0±7.6	98.7±9.6	95.0±5.0	95.7±5.9	94.1±4.3
control gr. (compare)	95.4±7.1	96.3±7.2	98.8±6.9	90.6±5.5	93.9±4.7	93.4±4.7
control gr. (diabetes)	85.3±6.68	269.7±102.0*	324.0±102.5*	248.0±144.0*	353.9±133.6*	249.7±108.7*
experimental gr. 1(2.90 mg/kg.bw.)	86.9±7.4	312.3±134..2*	118.0±75.1	225.2±93.6*	155.9±81.8	212.9±126.6*
experimental gr. 2(5.79 mg/kg.bw.)	87.1±8.8	280.1±131.1*	249.3±116.1*	218.9±99.2*	226.6±124.4*	186.2±108.1*
experimental gr. 3(11.43 mg/kg.bw.)	90.5±7.5	179.9±119.9*	244.3±104.1*	208.8±97.1*	214.2±102.5*	190.8±132.2*

All values are mean ± SD, * means p-value < 0.05

The blood sugar level of rats that received *Gynura divaricata* DC. Ethanol crude extract at dose 2.90 mg/kg.bw. Was not significantly different compared with the normal control group and compare control group on the first and third weeks, but significantly increased on the second and forth weeks ($p<0.05$). Both 5.79 mg/kg.bw. And 11.43 mg/kg.bw. Groups were shown to have the high blood sugar level with the significance

different comparing with the the normal control group and compare control group ($p<0.05$) for 4 weeks after receiving crude extract.

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Discussion

In this study, the ethanol crude extract with the dose of 2.9 mg/kg could decrease blood sugar of diabetic rats. The blood sugar level was not significantly different compared with the normal control group and compare control group within the 1st week of treatment and maintain it for one week. The fluctuation of blood sugar level is observed from the 2nd week to the end of experiment. It is possible that, the incomplete dissolution into the limit volume of control solvent, Tween 80, limits the crude molecules disintegration. It could be proposed that Gynura divaricata DC. Crude extract at the dose 2.9 mg/kg could be used as a supplement herbal for the diabetic patients. The mechanism of Gynura divaricata DC. Ethanol crude extract has not yet been proved, however. Recently, Wu,T. et.al. (2011) found the in vitro inhibitory effect of Gynura divaricata (L.) on amylase and α-glycosidase, the enzymes for metabolism of carbohydrate into glucose. They proposed that Gynura divaricata could be used for decreasing blood sugar in the type II Diabetic Melitus. Moreover, Gynura procumbens extract could inhibit Angiotensin-1 converting enzyme (ACE), which plays an important role on hypertension. Zhang,X.F. and B. K. Tan, (2000) found the ethanol extract of Gynura proccumbens decreased blood sugar level in diabetic rats by acting via the same mechanism of the biguanide, antihyperglycemic drugs.

Conclusion

The daily oral administration of *Gynura divaricata* DC. Ethanol crude extract in Streptozotozin induced diabetic rats at the dose of 2.9 mg/kg could decrease blood sugar level to a little higher than normal range within the 1st week of treatment. It is suggested that, *Gynura divaricata* DC. Ethanol crude extract could be used as supplement herbal to the antihyperglycemic drugs in the treatment of diabetic patients.

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