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Short Communication

Acute Toxicity Study and Elemental Analysis of Gasca N Herbal Product

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ABSTRACT

The biological effectiveness of any herbal medicinal product depends on its safety and efficacy. In recent years the world has witnessed an increased usage of herbal medicinal products, hence their potential toxicities and adverse effects is a matter of public health importance. The study aimed to assess the acute toxicity and to determine the level of essential elements and heavy metals in Gasca N herbal product. The acute toxicity study was done according to standard procedure, where Wistar rats were administered Gasca N herbal product at a single dose of 10 mg/kg, 100 mg/kg, and 1000 mg/kg (Phase I) then 1,600 mg/kg, 2,900 mg/kg and 5,000 mg/kg (Phase II). The concentration of heavy metals (Cadmium, Arsenic, Mercury, Lead and chromium) and that of important elements like Iron, Cobalt, Zinc, Vanadium and Copper were estimated using standard procedure with Microwave Plasma-Atomic Emission Spectrometry 4200.The results of the present study demonstrated that oral administration of Gasca N herbal product up to a dose of 5,000 mg/kg did not cause any mortality or adverse effect, suggesting that the product can be considered as practically nontoxic. Heavy metal concentrations in the herbal product were found to be as follows: Pb (0.0), Hg (0.0), Cd (0.76 µg/g), Cr (2.92 µg/g), and As (0.0). Gasca N herbal product has been shown to be non-toxic. The levels of heavy metals present in Gasca N herbal product fall within the permissible limits according to WHO permissible limits, thus does not pose a significant threat to human health.

Keywords: Acute toxicity, elemental, Gasca N, Herbal, Heavy Metals.

Introduction

The primary step in the assessment of any traditional medicine is the characterization of its toxic potential through acute oral toxicity screening. Based on this, the present study was aimed at evaluating the safety of Gasca N herbal product on acute oral administration and to determine the level of heavy metals therein.

Gasca N herbal formulation was developed from *Adansonia digitata* and *Hyphaenae thebaica* which serve as the major active ingredients. *Adansonia digitata* is a multi-purpose plant, which is used both as food and medicine. The seeds, leaves, roots, flowers, fruit pulp and bark of the plant are edible. The fruit was found to have high vitamin C content at 280 to 300 mg/100 g, compared to vitamin C content of 46 mg/100 g in oranges.[1] The fruit pulp is acidic, due to the presence of organic acids such as citric, tartaric, malic, succinic and ascorbic, with pH 3.3.[2] Protein content of baobab accounts for about one-fifth of dry matter in baobab fruit pulp (17%).[3] Baobab fruit pulp is a rich source of linoleic acid and α-linolenic acid which are essential for human nutrition.[3] The fruit pulp contains detectable levels of α-carotene and lutein[3], thiamine, riboflavin, and niacin.[4] The seed contains a relatively high amount of essential amino acids.[5-7] The fruit pulp has been found to have similar anti-inflammatory properties to phenylbutazone used as standard in rats.[8] The leaves, fruit-pulp and seeds have shown antiviral activity against influenza virus, herpes simplex virus and respiratory syncytial virus[9] and polio.[10] The plant has been reported to be used in folk medicine as an antipyretic or febrifuge to overcome fevers. Powdered seeds and fruit pulp have traditionally been used as an immunostimulant.[11] The plant has been reported to have anti-rheumatoid activity.[12]

The fruit of the *Hyphaene thebaica*, known as ginger bread, is considered a life-sustaining nutrient in desert areas, especially during periods of drought.[13] *Hyphaene thebaica* has an antioxidant activity due to the substantial amount of water-soluble phenolic contents of flavonoids within it. These contents represent conjugates of o-glycosides, which include quercetin, chrysoeriol, luteolin, and isorhamnetin H.[13,14] Moreover, *Hyphaene thebaica* is known to possess anti-inflammatory capacities due to its ability to inhibit cyclooxygenase (COX-1), an enzyme known to be involved in the inflammation.[15] Research on the fruit pulp has shown that it contains nutritional trace minerals, proteins and fatty acids, particular the nutritionally essential linoleic acid.[16] It has been reported that *Hyphaene thebaica* has been screened as a viable source of natural antioxidants including tocopherols, vitamin C, carotenoids and phenolic compounds.[16,17]

Hyphaene thebaica was reported to lower the blood pressure, when its biological activity was evaluated in rat feeding experiments.[18] TLC analysis of hot water extract of *Hyphaene thebaica* fruit showed the presence of saponins, coumarins, hydroxycinnamates, essential oils and flavonoids,[18] which act to prevent or reduce oxidative stress by scavenging free radicals.[19-21] The medicinal activity of many plants can be attributed to bioactive compounds, which could delay or inhibit the inception of degenerative diseases and increase life expectancy.[22]

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The has been several reported cases of adverse effects of herbal medicinal preparation consumption in developing countries sold by the traditional practitioners without proper prescription of dosage regimen or assessment of its toxic potential as many of these products contain considerable amount of toxic heavy metals such as arsenic, cadmium, lead and mercury.[23] These may cause various ailments like liver and kidney disease to mention a few. Contamination of herbal products with chemicals such as pesticides and heavy metals that are known to be injurious to health can be detrimental to human health. Most herbal contaminants can be traced back to the source of the herbal raw material, or methods and materials used in the preparation. In developing countries, where sales, regulation, importation, and manufacturing of herbal medicinal products are not subjected to rigorous scientific analysis in terms of safety and efficacy as is the case with conventional medication, heavy metals have been reported in some of these herbal medicinal products.[24,25] Heavy metals have been known to constitute a significant health risk to consumers when ingested via fluids, food or through other contaminants because they bioaccumulate in the body and are stored faster than they are excreted.[25,26] With the continued increase in the consumption of herbal medicinal products globally, it has become necessary to investigate its potential toxicity, in order to furnish the consumers as well as healthcare professionals with adequate information regarding the safety profile of these herbal products in order to have a clear understanding in making informed decision with the risks associated with the consumption of these herbal products.

Materials and Methods

Sample
Gasca N herbal product sample was obtained directly from the manufacturer at Greenleaf herbal product situated at no 5664 Yan dodo Hotoro Nassarawa local government area of Kano State.

Gasca N Herbal product
The product contains extract of *Hyphenae thebaica*, *Adansonia digitata,* and *Gum Acacia*

Experimental animals
Young healthy male Wistar rats of about 150 – 180 g body weight were used for the acute toxicity study in accordance with the local ethics committee of Bayero University Kano for use and care of animals in conformity with the NIH recommendations. The experimental animals were bred and obtained from the Department of Pharmacology, Ahmadu Bello University Zaria, Nigeria. The experimental animals were kept at room temperature between (23 - 25°C) and were exposed to 12 h/12 h light/dark cycle, the rats were fed with vitafeeds (Animalfeeds) diet and had access to water *ad libitum*. The experimental animals were maintained in the laboratory for a week to acclimatize before the commencement of the study.

Experimental design
Acute toxicity study
The acute toxicity study was conducted according to the method of Lorke.[27] In the first phase of the study, nine (9) rats were randomly assigned into 3 groups, forming 3 rats per group. Groups 1, 2 and 3 were administered orally with Gasca N herbal formulation after comminution into fine powder and diluted with distilled water at a dose of 10, 100 and 1000 mg/kg body weight, respectively. Absence of any morbidity and mortality after 72 hours led to the second phase of administration, where Gasca N was orally administered at a dose of 1900, 2600 and 5000 mg/kg body weight to group 1, 2 and 3, respectively. General clinical observations for morbidity and mortality were made after every 6 hours for 72 hours for any sign of changes in the skin and fur, eyes, respiratory system, nasal bleeding, wetness, paralysis and behavioral patterns were also observed to detect any signs of toxicity.

Heavy Metal and Mineral Analysis
Digestion of the sample
Precisely 5 g of the sample was weighed and dissolved in 10 mL of mixed acid (HNO_3/HCl in the ratio 1:3) for 12 h. The mixture was heated on a hot plate until the brown fumes changed to white. The mixture was allowed to cool, then followed by the addition of 10 mL distilled water and heated again. The mixture was then filtered into 50 mL volumetric flask after cooling. The final mixture was made up to 50 mL by addition of deionized water. Reagent blank was also prepared in the same way.

Instrumentation
Determination of Zinc (Zn), Copper (Cu), Lead (Pb), Iron (Fe), Molybdenum (Mo), Mercury (Hg), Silicon (Si), Vanadium (V), Cadmium (Cd), Nickel (Ni), Cobalt (Co), Manganese (Mn), Chromium (Cr), Aluminium (Al) and Arsenic (As) in Gasca N herbal medicinal product was carried out using Microwave Plasma-Atomic Emission Spectrometry 4200 (Agilent, USA). Automatic optimization of viewing position and nebulizer pressures was done using the Agilent MP Expert software. Manual sample introduction mode was used. All measurements were done in duplicate for the sample and standard solution.

Calibration, Background and Interference Corrections
Multi-element standard solutions were used for the analytical calibration of all the elements except for Arsenic. Due to Fe spectral interference on the As 234.984 nm emission line, a separate calibration solutions were used for As. For the background correction an auto-background correction feature of the MP Expert software was used. In order to correct and remove the iron interference on the determination of arsenic using the 234.984 nm line a Fast Linear Interference Correction (FLIC) method was used.

Results and Discussion

Evaluating the acute toxicity of any plant with potential therapeutic value is essential to establishing its safety profile. The safety profile must be established as a guide for the management of its applications and usage. Result of the present study showed that administration of Gasca N herbal product to experimental animals did not result in any morbidity or mortality up to an acute dose of 5000 mg/ kg bw of the herbal product. Mortality is the main criteria in assessing the acute toxicity (LD_{50}) of any herbal medicinal product as far as acute dose is concerned. Result of the present study clearly indicates that Gasca N herbal product has a 50% lethal dose (LD_{50}) above 5000 mg/kg, a dose that is considered as nontoxic. All the elements concentration found in Gasca N herbal product were within acceptable limit of intake. Nevertheless, high or low levels of these elements could cause serious problem to human health. Many of these elements are required within a specific limitation. Deficiencies of certain minerals, such as iron, magnesium, and chromium, etc. may cause impairment of normal metabolic function. Anemia is a condition characterized by low level of red blood cells. One of the most significant contributors, accounting for 50% of all causes of anemia is Iron deficiency anemia (IDA).[28] Anemia has been reported to be a critical indicator of cancer risk.[29] Anemia can affect the quality of life and has been found to shorten survival in people with cancer. Gasca N is a herbal medicinal product produced and use locally for the management of cancer. The concentration of iron in this product was found to be the highest among all the elements analyzed, this suggests that the product could as well be a good source of iron supplement for the management of iron deficiencies in cancer patients. Early development of oral and liver tumors has been associated with accelerated iron deficiency in animal models.[30,31] Iron deficiency has also been reported to increase the incidence of colon and duodenal tumors.[32] Previous studies have reported that iron deficiency anemia (IDA) alters immune activities including cellular and humoral immunity,[33-36] thus creating a microenvironment permissive for carcinogenesis. It was found out that the overall cancer risk was higher in IDA patients than in the general population.[37] The high concentration

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Table 1: General appearance and behavioral observations after administration of Gasca N

Observation	6 hrs	12 hrs	24 hrs	48 hrs	72 hrs
Behavioral patterns	Normal	Normal	Normal	Normal	Normal
Condition of fur	Normal	Normal	Normal	Normal	Normal
Subcutaneous swelling or lumps	Normal	Normal	Normal	Normal	Normal
Nasal bleeding	Normal	Normal	Normal	Normal	Normal
paralysis	Normal	Normal	Normal	Normal	Normal
Wetness and / or soiling of perineum	Normal	Normal	Normal	Normal	Normal
Breathing abnormalities	Normal	Normal	Normal	Normal	Normal
Diarrhea	Not observed	Not observed	Not observed	Not observed	Not observed
Tremors/Corner sitting	Not observed	Not observed	Not observed	Not observed	Not observed
Mortality	None	None	None	None	None

of iron in Gasca N herbal product can make it a good candidate for iron supplementation.

Chromium has been found to be an essential trace element for maintaining healthy body.[38] Daily mean intake for chromium has been reported to be around approximately 33 µg.[39] Previous studies have suggested the possibility of chromium supplementation in influencing glucose tolerance and insulin resistance in humans,[40,41] and rats.[42] *In vitro* experiments showed that chromium and insulin supplementation to animal tissues resulted in increased glucose oxidation to carbon dioxide and water, increased glycogenesis and conversion of glucose to lipids, as well as increased glucose utilization.[43] The presence of chromium in Gasca N herbal product could as well provide the needed chromium supplementation, because chromium has been reported in previous studies to influence glucose metabolism and Gasca N is a herbal product designed for use in patients with cancer, and cancer has been known to cause impairment of glucose metabolism, hence, chromium could as well play a role in providing a balance towards the restoration of altered glucose metabolism. The intake of chromium as a nutritional supplement includes; improved growth, increased muscle mass, improved reproductive function, boost in immune response.[44]

Vanadium is a very important element that has been reported to help in controlling the development of diseases such as cancer and diabetes. Gasca N has been found to contain a considerable amount of vanadium. Vanadium compounds have been reported to influence glucose and lipid metabolism.[45] Vanadium compounds have been reported to affect the levels of glucose, cholesterol and triacylglycerols, with no harmful side effects after prolonged administration.[46-50] Experiments performed with diabetic patients, confirmed the therapeutic effect of vanadium compounds on blood glucose levels with little side effects.[51] Vanadium compounds were also found to have an effect against cancer cells, contraction of blood vessels, enhancement of oxygen-affinity of hemoglobin and myoglobin.[52-54] The inhibition of the growth of human tumor colony formation has been reported to be effected by vanadium compounds.[55] Vanadium was found to reduce tumor size and incidences in various carcinogenic models.[56,57]

Vanadium compounds have also been shown to possess antineoplastic activity against rat liver tumors,[58] fluid and solid Ehrlich ascites tumor,[59] and TA3Ha murine mammary adenocarcinoma.[60] Bishayee and Chatterjee,[61] reported the antitumor activity of some vanadium compounds in animal model systems. The presence of vanadium in Gasca N herbal product points towards a positive effect in managing cancer patients.

Manganese (Mn) is an essential element necessary for metabolic function in the body which includes; healthy growth and development, activation of certain metalloenzymes, energy metabolism, immune function, nervous function, reproductive hormone function, and as antioxidant.[62,63] Gasca N herbal product has been found to have manganese at a concentration of 11.44 µg/g, and this shows that Gasca N can provide this essential element to the metabolic processes that requires it. Manganese has been reported to interact with pyruvate carboxylase, an essential enzyme necessary for the first step in gluconeogenesis to generate oxaloacetate.[64] Gasca N herbal product can thus be used to provide the needed amount of Mn that may be required for metabolic function. In addition, Mn has been found to play essential role in the regulation of cellular energy, bone and connective tissue growth, and blood clotting. Manganese is an essential cofactor for a variety of enzymes, including those involved in neurotransmitter synthesis and metabolism.[65] Aluminum has been used in medicine for years as an adjuvant in vaccines and an agent against pathological hyperhidrosis with a low side-effect profile.[66,67] No acute toxic effect of dietary intake of aluminum has been reported in the general population in recent years due to its low acute toxicity.[39] The present study reports aluminum concentration in Gasca N herbal product to be 25.56 µg/g, which is significantly below the biological tolerance level for occupational exposure, which has been set to be 50 µg/g and the limit was below the tolerable weekly intake (TWI) of the European Food Safety Authority (EFSA) of 1 mg aluminum/kg body weight (BW) in a 60 kg adult.[68]

Table 2: Elemental Analysis of Gasca N Herbal Formulation

Elements	Concentration (µg/g)
Zn	0.0 ± 0.0
Cu	3.16 ± 0.40
Pb	0.0 ± 0.0
Fe	129.84 ± 3.80
Mo	0.0 ± 0.0
Hg	0.0 ± 0.0
Si	0.0 ± 0.0
V	2.04 ± 0.30
Cd	0.76 ± 0.07
Ni	2.2 ± 0.20
Co	1.28 ± 0.06
Mn	11.44 ± 1.30
Cr	2.92 ± 0.01
Al	25.56 ± 4.10
As	0.0 ± 0.0

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Conclusion

The finding of the acute oral toxicity study revealed that Gasca N herbal product can be said to be nontoxic at an acute dose of 5,000 mg /kg bw, suggesting Gasca N to be a practically nontoxic herbal drug. Many of the elements found in Gasca N herbal product are within the recommended limit of intake and have also been found to exhibit function in the management of cancer.

Conflict of Interest

The authors declare no conflict of interest

Author's Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this work will be borne by them.

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References

1. Vertuani S, Braccioli E, Buzzoni V, Manfredini S. Antioxidant capacity of *Adansonia digitata* fruit pulp and leaves. Acta Phytother. 2002; 86:2-7.
2. Nour AA, Magboul BI, Kheiri NH. *Chemical composition of baobab fruit (Adansonia digitata L.).* Trop Sci. 1980; 22:383-388.
3. Sena LP, VanderJagt DJ, Rivera C, Tin AC, Muhamadu I, Mahamadou O, Millton M, Pastuszyn A, Glew RH. Analysis of nutritional components of eight famine foods of the Republic of Niger. Plant Food Hum Nutr. 1998; 52:17-30.
4. Becker B. The contribution of wild plants to human nutrition in the Ferlo (Northern Senegal). Agroforestry Syst. 1983; 1:257-267.
5. Odetokun SM. The nutritive value of Baobab fruit (Adansonia digitata). La Rivista Italiana delle Sostanze Grasse. 1996; 73:371-373.
6. Osman MA. Chemical and Nutrient Analysis of Baobab (Adansonia digitata) Fruit and Seed Protein Solubility. Plant Food Hum Nutr. 2004; 59:29-33.
7. Glew RH, VanderJagt DJ, Lockett C, Grivetti LE, Smith GC, Pastuszyn A, Millson M. Amino Acid, Fatty Acid and Mineral Composition of 24 Indigenous Plants of Burkina Faso. J Food Comp Analys. 1997; 10:205-217.
8. Ramadan A, Harraz FM, El-Mougy SA. Anti-inflammatory, analgesic and antipyretic effects of the fruit pulp of *Adansonia digitata*. Fitoterapia. 1993; 65:418-422.
9. Vimalanathan S, Hudson JB. Multiple inflammatory and antiviral activities in *Adansonia digitata* (Baobab) leaves, fruits and seeds. J Med Plants Res. 2008; 3:576-582.
10. Al-Qarawi AA, Al-Damegh MA, El-Mougy SA. Hepatoprotective Influence of *Adansonia digitata* Pulp. J Herbs Spices Med Plants. 2003; 10:1-6.
11. Anani K, Hudson JB, de Souzal C, Akpagana K, Tower GHN, Amason JT, Gbeassor M. Investigation of medicinal plants of Togo for antiviral and antimicrobial activities. Pharm Biol. 2000; 38:40-45.
12. Thiyagarajan V, Muthusamy P, Jayshree N, Vijaya B, Raj K. A Review on *Adansonia digitata*- Potential Herb. Research J Pharmacogn Phytochem. 2015; 7(1):1-4.
13. Hsu B, Coupar IM, Ng K. Antioxidant activity of hot water extract from the fruit of the Doum palm, Hyphaene thebaica. Food Chem. 2006; 98:317–328.
14. Abdel-Rahim EA, El-Beltagi H, Fayed A. Comparative studies on the influences of Juniperus phoenicea and Hyphaena thebaica as hypoglycemic factors in diabetic rats. J Adv Food Sci. 2011; 33:128–132.
15. Shehu B, Gidado A, Buratai L. Hypoglycaemic, hypolipidaemic and possible toxicity of the Methanolic fruit pulp extract of hyphane thebaica (L) Mart in alloxan--induced diabetic rats. J Med Appl Biosci. 2014; 6:1-10.
16. Cook JA, VanderJagt DJ, Pastuszyn A, Mounkaila G, Glew RS, Millison M, Kilani S, Sghaier MB, Limem I, Bouhlel I, Boubaker J, Bhouri W, Skandrani I, Neffatti A, Ammar RB, Dijoux-Franca, MG, Ghedira K, and Chekir-Ghedira L. In vitroevaluation of antibacterial, antioxidant, cytotoxic and apoptotic activities of the tubers infusion and extracts of Cyperus rotundus. Biores Technol. 2008; 99:9004–9008.
17. Yang JH, Lin HC, Mau JL. Antioxidant properties of several commercial mushrooms. Food Chem. 2002; 77:229–235.
18. Betty Hsu, Coupar MI, Ken Ng. Antioxidant activity of hot water extract from the fruit of the Duom palm "*Hyphaene thebaica*". Food Chem. 2006; 98:317–328.
19. Eldahshan OA, Ayoub NA, Singab AB, Al-Azizi MM. Potential superoxide anion radical scavenging activity of doum palm (*Hyphaene thebaica* L.) leaves extract. Rec Nat Prod. 2008; 2:83–93.
20. Jeong JB, Ju SY, Park JH, Lee JR, Yun KW, Kwon ST, Lim JH, Chung GY, Jeong HJ. Antioxidant activity in essential oils of *Cnidium officinale makino* and *Ligusticum chuanxionghort* and their inhibitory effects on DNA damage and apoptosis induced by ultraviolet B in mammalian cell. Cancer Epidemiol. 2009; 33:41–46.
21. Vassalle C, Petrozzi L, Bollo N, Andressi MG, Zucchelli GC. Oxidative stress and its association with coronary artery disease and different atherogenic risk factors. J Int Med. 2004; 256:308-315.
22. Jagadish LK, Krishnan VV, Shenbhagaraman R, Kaviyarasan V. Comparative study on the antioxidant, anticancer and antimicrobial property of Agaricus bisporus Imbach before and after boiling. Afr J Biotechnol. 2009; 8:654-661.
23. Martena MJ, Van Der Wielen JC, Rietjens IM, Klerx WN, De Groot HN, Konings EJ. Monitoring of mercury, arsenic and lead in traditional Asian herbal preparations on the Dutch market and estimation of associated risks. U.S Nat Lib Med Contr Expo Risk. 2010; 27:190-205.
24. Barnes J. Quality, efficacy and safety of complementary medicines: fashions, facts and the future. Part1: Regulation and quality. Br J Clin Pharmacol. 2003; 55:226-233.
25. Zaleska A. Heavy metals: health and environmental effects of some heavy metals (concentration on RoHS Directive), Gdańsk. Chemical Faculty, Gdańsk University of Technology PG Press. 2008. 1- 27 p.
26. Phillips S and Balge M. Heavy metal toxicity, Texas (SA). New Fields, 2007. 1-30 p.
27. Lorke D. Anew approach to practical toxicity testing. Arch Toxicol. 1983; 54(4):275-287
28. de Benoist B ME, Egli I, Cogswell M, eds. Worldwide prevalence of anemia WHO Global Database on Anaemia Geneva, Switzerland: WHO press, 2008. 1–40. 1993-2005.
29. Knight K, Wade S, Balducci L. Prevalence and outcomes of anemia in cancer: a systematic review of the literature. Am J Med. 2004; 116 Suppl 7A:11s–26s.
30. Vitale JJ, Broitman SA, Vavrousek-Jakuba E, Rodday PW, Gottlieb LS. The effects of iron deficiency and the quality and quantity of fat on chemically induced cancer. Adv Exp Med Biol. 1977; 91:229-242.

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31. Prime SS, MacDonald DG, Rennie JS. The effect of iron deficiency on experimental oral carcinogenesis in the rat. Br J Cancer. 1983; 47(3):413–418.
32. Jagadeesan V, Rao NJ, Sesikeran B. Effect of iron deficiency on DMH-induced gastrointestinal tract tumors and occurrence of hepatocyte abnormalities in Fischer rats. Nutr Cancer. 1994; 22(3):285–91.
33. Attia MA, Essa SA, Nosair NA, Amin AM, El-Agamy OA. Effect of iron deficiency anemia and its treatment on cell mediated immunity. Indian J Hematol Blood Transfus. 2009; 25(2):70-77.
34. Tang YM, Chen XZ, Li GR, Zhou RH, Ning H, Yan H. [Effects of iron deficiency anemia on immunity and infectious disease in pregnant women]. Wei sheng yan jiu Journal of hygiene research. 2006; 35(1):79–81.
35. Das I, Saha K, Mukhopadhyay D, Roy S, Raychaudhuri G, Chatterjee M, Mitra PK. Impact of iron deficiency anemia on cell-mediated and humoral immunity in children: A case control study. J Nat Sci Biol Med. 2014; 5(1):158–163.
36. Ekiz C, Agaoglu L, Karakas Z, Gurel N, Yalcin I. The effect of iron deficiency anemia on the function of the immune system. Hematol J. 2005; 5(7):579–583.
37. Hung N, Shen C, Hu Y, Hu L, Yeh C, Teng C, Kuan A, Chen S, Chen T and Liu C. Risk of Cancer in Patients with Iron Deficiency Anemia: A Nationwide Population-Based Study**.** PLoS One. 2015; 10(3):e0119647.
38. Mertz W. Biological role of chromium. Federation Proceedings. 1967; 26:186–193.
39. WHO (World Health Organization) Aluminium. International programme on chemical safety (IPCS), environmental health criteria 194. Genf: WHO 1997. www.inchem.org/documents/ehc/ehc/ehc194.htm (la st accessed on 21 July 2017)
40. Anderson RA. Chromium in the prevention and control of diabetes. Diabetes and Metabolism. 2000; 26:22–27.
41. Tuzcu A, Bahceci M, Dursun M, Parmaksiz Y, Ertem M, Dalgic A, Turgut C, Kale E. Can long-term exposure to chromium improve insulin sensitivity in chromium mine workers? J Trace Elem Exp Med. 2004; 17:55–63.
42. Kim DS, Kim TW, Kang JS. Chromium picolinate supplementation improves insulin sensitivity in Goto-Kakizaki diabetic rats. J Trace Elem Med Biol. 2004; 17:243–247.
43. Anderson RA. Nutritional factors influencing the glucose/insulin system: Chromium. J Am Coll Nutr. 1997; 16:404–410.
44. Pechova A and Pavlata L. Chromium as an essential nutrient: A review. Vet Med. 2007; 52: (1):1–18
45. Nakai M, Watanabe H, Fujiwara C, Kakegawam H, Satoh T, Takada J, Matsushita R, Sakurai H. Mechanism of insulin-like action of vanadyl sulfate: studies on interaction between rat adipocytes and vanadium compounds. Biol Pharm Bull.1995; 18:719–725.
46. Yanardag R, Bolkent S, Karabulut-Bulan O, Tunali S. Effects of vanadyl sulfate on kidney in experimental diabetes. Biol Trace Elem Res.2003; 95:73–85.
47. Koyuturk M, Tunali S, Bolkent S, Yanardag R. Effects of vanadyl sulfate on liver of streptozotocin-induced diabetic rats. Biol Trace Elem Res 2005; 104:233–247.
48. Niu Y, Liu W, Tian C, Xie M, Gao L, Chen Z, Chen X, Li L. Effects of bis(alpha-furancarboxylato)oxovanadium(IV) on glucose metabolism in fat-fed/streptozotocin-diabetic rats. Eur J Pharmacol. 2007; 572:213–219.
49. Wei D, Li M, Ding W. Effect of vanadate on gene expression of the insulin signaling pathway in skeletal muscle of streptozotocininduced diabetic rats. J Biol Inorg Chem. 2007; 12:1265–1273.
50. Li M, Smee JJ, Ding W, Crans DC. Anti-diabetic effects of sodium 4-amino-2,6-dipicolinatodioxovanadium(V) dihydrate in streptozotocin- induced diabetic rats. J Inorg Biochem. 2009; 103:585–589.
51. Thompson KH, Lichter J, LeBel C, Scaife MC, McNeill JH, Orvig C. Vanadium treatment of type 2 diabetes: a view to the future. J Inorg Biochem 2009; 103:554–558.
52. Poucheret P, Verma S, Grynpas MD, McNeill JH. Vanadium and diabetes. Mol Cell Biochem. 1998; 188:73–80.
53. Rehder D. Structure and function of vanadium compounds in living organisms. BioMetals 1992; 5:3–12.
54. Thompson KH, Leichter J, McNeil JH. Studies of vanadyl sulfate as a glucose-lowering agent in STZ diabetic rats. Biochem Biophys Res Commun. 1993; 97:1549–1555.
55. Hanauske U, Hanauske AR, Marshall MH, Muggia VA. Biphasic effect of vanadium salts on in vitro tumor colony growth. Int J Cell Clon.1987; 5:170–178.
56. Basak R, Chatterjee M.. Combined supplementation of vanadium and 1-, 25-dihydroxyvitamin D3 inhibit placental glutathione *S*-trasferase positive foci in rat liver carcinogenesis. Life Sci. 2000; 68:217–231.
57. Bishayee A, Roy S, Chatterjee M. Characterization of selective induction and alteration of xenobiotic blotransforming enzymes by vanadium during diethylnitrosamine-induced chemical rat liver carcinogenesis. Oncol Res.1999; 111:41–53.
58. Bishayee A, Chatterjee M. Inhibition of altered liver cell foci and persistent nodule growth by vanadium diethyl nitro samine-hepatocarcinogenesis in rats. Anticancer Res.1995a; 15:455–462.
59. Harding MM and Mokdsi G. Antitumor metallocenes: structure-activity studies and interactions with biomolecules. Curr Med Chem. 2000; 7:1289–1303.
60. Murthy MS, Rao LN, Kuo LY, Toney JH, Marks TJ. Anti-tumor and toxicologic properties of the organometallic anti-cancer agent vanadocene dichloride. Inorg Chem Acta 2000; 152:117–124.
61. Bishayee A and Chatterjee M. Inhibitory effect of vanadium on rat liver carcinogenesis initiated with diethyl nitrosamine and promoted by phenobarbital. Br J Cancer.1995b; 71:1214–1220.
62. ATSDR (Agency of Toxic Substances and Disease Registry), Toxicological Profi le for Manganese, U.S. Department of Health and Human Services Public Health Service, 2000.
63. IOM, Dietary Reference Intakes: Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc, Ed Institute of Medicine, National Academy Press, Washington DC, 2011.
64. Erikson KM and Aschner M. Neurochem. Int 2003; 43:475–480.
65. Fong NM, Jensen TC, Shah AS, Parekh NN, Saltiel AR, Brady MJ. J Biol Chem. 2000; 275: 35034–35039.
66. Paul-Ehrlich-Institut. Sicherheits bewertung von Aluminium in Therapie allergenen. www.pei.de/DE/arzneimittelsicherheit-vigilanz/archiv- sicherheitsinformationen (last accessed on 21 July 2017)
67. Deutsche Dermatologische Gesellschaft. Definition und Therapie der primären Hyperhidrose. S1-Leitlinie vom 15.1.2012. AWMF-Register Nr. 013/059. www.awmf.org/leitlinien (last accessed on 21 July 2017)
68. Klotz K, Weistenhöfer W, Neff F, Hartwig A, van Thriel C and Drexler H. The Health Effects of Aluminum Exposure**.** Dtsch Arztebl Int. 2017; 114(39): 653–659.

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