O‘ZBEKISTON RESPUBLIKASI

OLIY TA’LIM, FAN VA INNOVATSIYALAR VAZIRLIGI

FARG‘ONA DAVLAT UNIVERSITETI

FarDU. ILMIY XABARLAR-

1995-yildan nashr etiladi
Yilda 6 marta chiqadi

— 3-2023 —

НАУЧНЫЙ ВЕСТНИК. ФерГУ

Издаётся с 1995 года
Выходит 6 раз в год

UDK: 615.322:582.931 **DOI: 10.56292/SJFSU/vol29_iss3/a171**

DALACHOY TARKIBIDAGI VITAMINLAR MIQDORINI ANIQLASH VA ULARNING ORGANIZMGA TA'SIRI

ОПРЕДЕЛЕНИЕ КОЛИЧЕСТВА ВИТАМИНОВ, СОДЕРЖАЩИХСЯ В ЗВЕРОБОЕ, И ИХ ВЛИЯНИЯ НА ОРГАНИЗМ

DETERMINATION OF THE QUANTITY OF VITAMINS CONTAINED IN HYPERICUM PERFORATUM

Ibrohimjon Rahmonovich Asqarov[1], Go'yoxon Alidjonovna Mo'minova[2]

[1]Ibrohimjon Rahmonovich Asqarov − Andijon davlat universiteti, Kimyo kafedrasi professori, O'zbekistonda xizmat ko'rsatgan ixtirochi, O'zbekiston TABOBAT Akademiyasi raisi

[2]Go'yoxon Alidjonovna Mo'minova − Andijon davlat tibbiyot instituti, Biologik kimyo kafedrasi dotsenti, biologiya fanlari bo'yicha falsafa doktori (PhD)

Annotatsiya

Hozirgi kunga kelib, o'simliklardan tayyorlangan tabiiy-dori vositalariga qiziqish va ehtiyoj ortib boryapti. Butun dunyoda bemorlarni davolashda o'simliklardan tayorlangan tabiiy-dori vositalar va oziq-ovqat qo'shilmalaridan foydalanish Yevropa davlatlarida yuqori ko'rsatkichlarni tashkil qiladi. Ayniqsa Germaniyada davolashda o'simliklardan foydalanish organizmga sintetik dori vositalaridan ko'ra foydaliroq ta'sirga ega ekanligini allaqachon tushunib yetishgan. Osiyoda joylashgan yirik rivojlangan davlatlar Yaponiya va Xitoyning tibbiyotda o'simliklardan keng foydalanishi hechkimga sir emas. Shuning uchun biz dalachoydan oziq-ovqat qo'shilmasi yaratish maqsadida, tarkibidagi vitaminlarni suyuqlik xromatografiyasi usuli yordamida aniqlandi. Tajriba davomida Dalachoy tarkibida B1,B2,B3,B6 va B9 vitaminlari aniqlandi. Antioksidant vitaminlardan vitamin Sning aniqlanishi dalachoyni teri regeneratsiyasida va qarish jarayonlarini oldini olishda ijobiy ta'siri borligidan dalolatdir.

Аннотация

В настоящее время возрастает интерес и потребность в натуральных лекарственных средствах, изготовленных из растений. Во всем мире использование растительных лекарственных средств и пищевых добавок при лечении пациентов является самым высоким в европейских странах. Тем более в Германии уже поняли, что использование растений в лечении более благотворно влияет на организм, чем синтетические препараты. Не секрет, что крупные развитые страны Азии, Япония и Китай широко используют растения в медицине. Поэтому, чтобы создать пищевую добавку из зверобоя, содержащиеся в ней витамины определяли методом жидкостной хроматографии. В ходе эксперимента в зверобое были обнаружены витамины B1, B2, B3, B6 и B9. Выявление витамина С среди витаминов-антиоксидантов свидетельствует о положительном влиянии зверобоя на регенерацию кожи и профилактику процессов старения.

Abstract

Nowadays there is a growing interest and demand for natural medicines made from plants. Worldwide, the use of herbal medicines and nutritional supplements in the treatment of patients is the highest in European countries. Moreover, in Germany they have already realized that the use of plants in treatment has a more beneficial effect on the body than synthetic drugs. It is no secret that the large developed countries of Asia, Japan and China widely use plants in medicine. Therefore, in order to create a nutritional supplement from Hypericum perforatum, the vitamins contained in it were determined by liquid chromatography. During the experiment, vitamins B1, B2, B3, B6 and B9 were found in Hypericum perforatum. The identification of vitamin C among antioxidant vitamins indicates a positive effect of Hypericum perforatum on skin regeneration and prevention of aging processes.

Kalit so'zlar: *Dalachoy, oziq-ovqat qo'shilmasi, tabiiy-dori vositalar, vitaminlar, ateroskleroz, qandli diabet, antioksidantlik, rak, xolesterin.*

Ключевые слова: *Зверобой, пищевая добавка, натуральное лекарство, витамины, атеросклероз, сахарный диабет, антиоксидант, рак, холестерин.*

Key words: *Hypericum perforatum, dietary supplement, natural medicine, vitamins, atherosclerosis, diabetes mellitus, antioxidant, cancer, cholesterol.*

KIRISH

Giperxolesterolemiya va ateroskleroz butun dunyoda o‘limning asosiy sababchisi bo‘lib qolmoqda. Yevropada 2 mln.dan ortiq odamda aterosleroz aniqlangan bo‘lib, davolash uchun esa bir yilda 192 milliard yevro sarflangan. Giperxolesterolemiyaning kichik yoshdan rivojlanishi, yosh bolalarda lipid skriningi o‘tkazishga ehtiyojni orttirib yubordi. Dietaterapiya zichligi past lipoproteinlar va shu orqali xolesterin miqdorini kamaytirish va yurak infarkti xavfini kamaytirish imkonini beradi. Vitaminga boy mahsulotlar bilan yurak–qon tomir kasalliklarini davolash yoki oldini olish barcha rivojlangan davlatlarda keng rivojlangan[1;2].

ADABIYOTLAR TAHLILI VA METODLAR

Vitamin B1 qandli diabetda juda muhim ahamiyatga ega. Qonda qand miqdori keskin oshganda tiamin qo‘llanilishi yuqori samara beradi. 1996-yilda o‘tkazilgan klinik va eksperimental tajribalar orqali tiaminni qandli diabetda qo‘llash ijobiy natija berishini ko‘rsatib berishdi.Chunki tiamin transketolaza fermentining kofermenti bo‘lib, miqdorini ortishi bu ferment faolligi ortishiga sabab bo‘ladi. Transketolaza fermenti glyukozaning pentozafosfat yo‘li asosiy fermenti hisoblanadi[3;4;5].

Tiaminni kofermentlik funksiyasi 1937-yilda kashf qilingan bo‘lib, bu vitamin piruvat degidrogenaza kompleksi tarkibiga kirishi aniqlangan[6]. Hozirgi kunda tiamindifosfat kofermentlik qiluvchi 28 ta ferment kashf qilingan[7]. Vitamin B1ning yetishmasligi nafaqat sensorno-motor polinevropatiyani keltirib chiqaradi.Balki

biventrikulyar yurak yetishmovchilikni ham sababchisi bo‘lishi mumkin[8]. Vitamin B2 suvda eruvchi vitaminlar guruhiga mansub bo‘lib, termostabil vitamin bo‘lgani uchun ham ovqat tayyorlanish vaqtida parchalanib ketmaydi. Ammo uzoq vaqt quyosh nurining ta’sirida parchalanishi mumkin. Organizmga yuqori miqdorda tushishi, uni to‘planishiga sabab bo‘la olmaydi. Chunki ortiqcha miqdori siydik tarkibida chiqib ketadi[9;10].

Energiya almashinuvi jarayonida ishtirok etuvchi FAD, FMN kabi kofaktorlar tarkibiga kiradi. Vitamin B2 yurak ishemiyasi, qandli diabet va stenokardiyada rivojlanuvchi oksidlanishli stressni oldini oluvchi asosiy vositalardan biri hisoblanadi[11]. Zou va boshq. lar (2015y) drozofila pashshasida antioksidant fermentlarni vitamin B2 ta’sirida aktivlanishini va bu vitamin ta’sirida ularda umrning uzayishi va ko‘payish jarayoning jadallashishini kuzatishdi[12]. Vitamin B2 kislorodning aktiv formalari miqdorini kamaytirishi kuzatilgan[13]. Vitamin B2 katalaza, superoksidismutaza va glutationperoksidaza kabi antioksidant fermentlar konsentratsiyasiga ta’sir etadi. Riboflavin glutationperoksidaza va ksantinoksidaza ferment sistemalarining bir qismi hisoblanadi. Chunki glutationni qaytarilishida riboflavin FAD ko‘rinishida qatnashadi[14]. Nikotin kislota 1955-yilda qonda xolesterin miqdorini kamaytirishi isbotlangan va hozirgi kunda dislipidemiya, gipertriglitseridemiya va aterosklerozni oldini olish uchun ishlatiladi[15; 16]. Hayvonlarda vitamin B3 yetishmovchiligi tufayli NAD+ miqdori kamayadi. Natijada somatik hujayralarda mutatsiyalar soni oshib, rak paydo bo‘lish xavfi oshadi[17]

Vitamin B6 150 dan ortiq reaksiyalarda kofaktor vazifasini bajaradi. Organizmda yallio‘lanish jarayoni aniqlanganda vitamin B6 miqdori plazmada kamligi aniqlangan. Plazma tarkibidagi vitamin B6 ning miqdori yurak-qon tomir kasalliklarida, ayrim rak kasalliklarida o‘zgarishi mumkun. Vitamin B6 ni qabul qilish immun tizim funksiyasini yaxshilanishini ta’minlaydi[18;19]. Yaponiyada 40-59 yoshdagilar o‘rtasida o‘tkazilgan kuzatishlarda 40803 kishi qatnashdi va bular ichida v6 vitaminini sutkasiga 1.3 mg dan 1.6 mg gacha qabul qilganlarda yurak infarkti xavfi kamayishi kuzatilgan[20]. Adabiyotlar o‘rganib chiqilganda, B6 vitaminini qo‘shimcha sifatida berilganda qon bosimini tushirishi aniqlangan[21;22;23].

Coronary Health Project va shu kabi boshqa proyektlar vitamin B6 ning qabul qilinishini ko‘paytirish ateroskleroz va yurak ishemiyasi kasalliklarini kamayishini ko‘rsatdi[24;25].

Qandli diabet kasalligi asoratlaridan biri bu ateroskleroz bo‘lib, vitamin B6 endotelial disfunksiyani oldini olishi qator adabiyotlarda ko‘rsatib o‘tilgan[26;27]. Qandli diabetlarga bu vitaminni berish orqali 3.5-9.7%gacha aterosklerozni oldi olinishi mumkinligi ko‘rsatib berilgan[26]. B9 vitamini yetishmovchiligi bevosita qon-tomir kasalliklarini keltirib chiqaradi[28] va reproduktiv salomatlikka ta’sir etadi. Bu vitamin qator aminokislotalar, jumladan, metionoin, sistein, serin va glitsin sintezida muhim ahamiyatga ega[29]. AQSH da 15% ayollarda surunkali B9 vitamini yetishmovchili aniqlangandan so‘ng 1998-yildan boshlab non mahsulotlari B9 vitamini bilan boyitilishni boshladi[30]. Chunki homilador ayollarda vitamin B9 yetishmovchiligida, tuo‘ilgan chaqaloqlarda nerv trubkasi defekti nuqsoni bilan tuo‘ilgan. Agar folat kislota yetishmovchiligi oldi olinsa, 50-75% gacha bu nuqson oldini olishga erishish mumkun[31;32;33;34].

Vitamin C antioksidant vitaminlardan biri bo‘lib, uning yetishmasligi va oqibatlari qadimdan ma’lum bo‘lsada haligacha hattoki rivojlangan davlatlarda ham bu vitaminni yetishmasligi ko‘p uchraydi. Lekin surunkali vitamin C yetishmasligi oqibatlari singa bilangina chegaralanib qolmaydi. O‘tkazilgan klinik tadqiqotlar bu vitaminni yetishmasligi nafaqat singa, balki surunkali charchoq hissi va depressiyani keltirib chiqarishini ko‘rsatdi[35;36]. Olimlar vitamin C miqdori insult va diastolik qon bosimi rivojlanishi kuchli bashoratchisi ekanini aniqlashdi[37] .

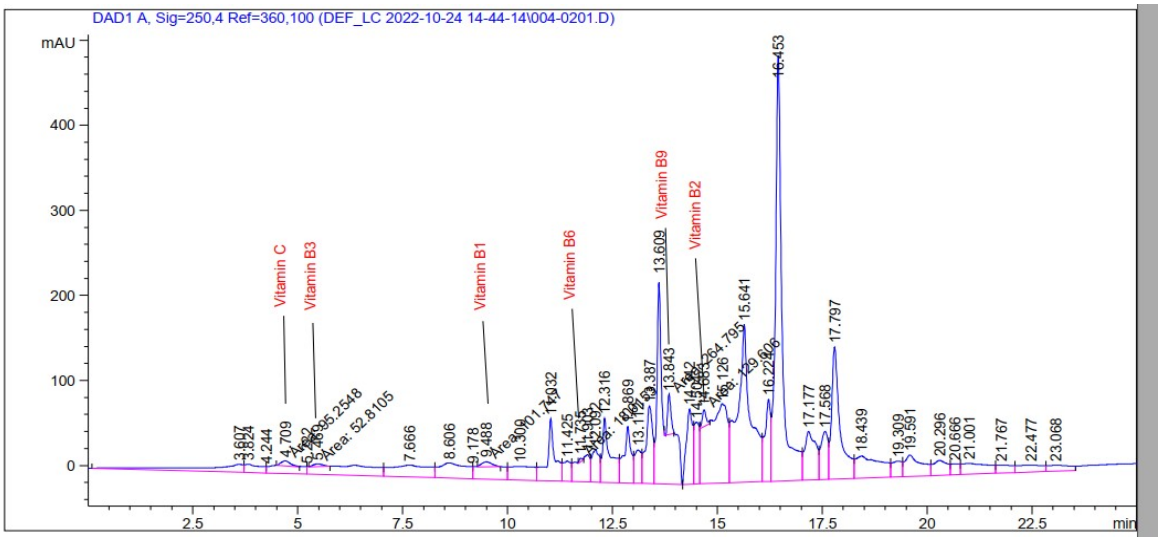
Adabiyotlar tahlilidan ko‘rinib turibdiki, Vitamin B1,B2,B3 B6 va vitamin C ning yetishmasligi yetishmasligi yurak-qon tomir kasalliklarini rivojlanishiga bevosita ta’sir etadi. Yurak-qon tomir kasalliklari qandli diabet kasalligi oqibatida ham ikkilamchi kasallik sifatida paydo bo‘lishi mumkun. Shuning uchun ham biz dalachoy o‘simligi tarkbidagi vitaminlarni aniqlashni maqsad qildik.

Tadqiqot metodologiyasi. Dalachoydan tayyorlangan namunalar tarkibidagi B1 (47858), B2 (47864), B6 (80823-50MG), B12 (PHR1234-1), C (47863) va PP (47865-U) katalog raqamdagi vitaminlari (Sigma Aldrich Germaniya)dan keltirilgan standart namunalar asosida sifat va miqdor ko‘rsatkichlarini Yaponiyada (Shimadzu) ishlab chiqarilgan YSSX LC 2030 C 3D Plus qurilmasi PDA detektori yordamida 260, 290 va 361 nm to‘lqin uzunligi tanlangan holatda aniqladi. Qo‘zg‘almas faza sifatida C18 250x4,6 mm 5 µm Precisely (Perkin Elmer) AQSH kalonkadan, foydalanildi. Vitaminlar analizi amalga oshirishda ko‘chma faza sifatida sirka kislotasining 0,5 %li eritmasi A faza va atsetonitril B faza o‘zgaruvchan rejim asosida amalga oshirildi.

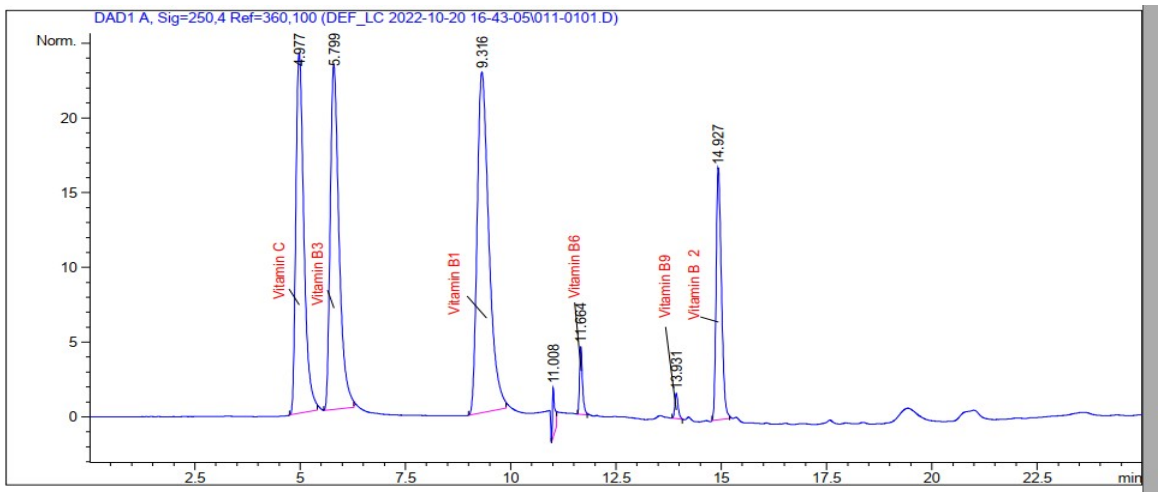
Vaqt	A faza % Sirka kislotasining suvdagi 0,5 % li eritmasi	B faza % Asetonitril
1	96	4
4	90	10
8	85	15
12	60	40
14	Stop	

TAHLIL VA NATIJALAR

Oqim tezligi 1 ml/minda, termostat harorati 40^0C da ineksiya qilingan namuna hajmi 10 mkl, analiz vaqti 14 minutda amalga oshirildi va quyidagicha xromatogrammalar olindi.



1-rasm B_1, B_2, B3,B_6 ,B9 va C vitaminlarning Dalachoyning standart namunlaridan olingan xromatogrammasi



2-rasm B_1, B_2, B3,B_6 ,B9 va C vitaminlarning Dalachoyning ishchi namunlaridan olingan xromatogrammasi

Namunaning ekstraksiyasi quyidagicha amalga oshiriladi: Bunda keltirilgan namunadan 2 g (FA220 4N) analitik tarozida 0,001 mg aniqlikda o‘lchab olindi. So‘ngra 20 ml 0,1 N li xlorid kislotasi eritmasiga solinib, 40^0C haroratgda 15 min davomida magnit aralashtirgichda aralashtirildi. Olingan eritma xona haroratigacha sovitilib, 10 minut davomida 12000 ayl/daq semtrafuga qilindi va 0,45 μm filtrda filtrlab olinib vialga joylashtirildi va quyidagicha natijalar olindi.

Витамины	**Дала чой**
	Концентрация мкг/гр
B-1	0,1117
B-2 (B-12)	2,1114
B-6	0,4431
B-9	12,842
PP B-3	0,0805
Витамин С	0,127

NATIJALAR VA MUHOKAMA

Dalachoy tarkibida vitamin B1, B2, B3,vitamin B6,B9 va vitamin C lar aniqlandi. Vitamin B1 0,1117 mkg/gr B2 2,1114 mkg/gr,vitamin B3 0,0805 mkg/gr, vitamin B6 0,4431 mkg/gr, vitamin B9 12,842 mkg/gr va vitamin C 0,127 mkg/gr. Tajriba natijalaridan ko‘rinib turibdiki, dalachoy vitaminlarga boy o‘simlik bo‘lib, ayniqsa tarkibida qon-tomir kasalliklariga bevosita ta’sir etuvchi vitaminlardan vitamin B6 va vitamin B9 miqdori boshqa vitaminlardan ustunlik qiladi. Kuchli antioksidant vitamin hisoblangan vitamin C miqdori ham tiaminga nisbatan 1,14 marta, nikotin kislotaga nisbatan 1,58 marta ko‘pligini aniqladik.

XULOSA

Dalachoy tarkibida aniqlangan vitaminlar qon-tomir, rak, qandli diabet, onkologik kasalliklar va depressiyani oldini olishda muhim rol o‘ynaydi. Dalachoyning vitaminlarga boyligi gipertoniya,giperxolesterinemiya, giperlipidemiya, yallig‘lanish va trombozda qo‘llash mumkunligini ko‘rsatdi. Shuning uchun ham vitaminlarga boy bo‘lgan dalachoy o‘simligidan bezarar, yon ta’sirlardan holi oziq-ovqat qo‘shilmasi yaratish maqsadga muvofiq.

ADABIYOTLAR RO‘YXATI

1. Daniels SR, Gidding SS, de Ferranti SD. Pediatric aspects of familial hypercholesterolemias: recommendations from thye National Lipid Association Expert Panel on Familial Hypercholesterolemia. J Clin Lipidol. 2011;5:S30–S37. doi: 10.1016/j.jacl.2011.03.453. [PubMed] [CrossRef] [Google Scholar]
2. McNeal CJ, Dajani T, Wilson D, Cassidy-Bushrow AE, Dickerson JB, Ory M. Hypercholesterolemia in Youth: Opportunities and obstacles to prevent premature atherosclerotic cardiovascular disease. Curr Atheroscler Rep. 2010;12:20–28. doi: 10.1007/s11883-009-0072-0. [PubMed] [CrossRef] [Google Scholar]
3. Booth A. A., Khalifah R. G., Hudson B. G. Thiamine pyrophosphate and pyridoxamine inhibit the formation of antigenic advanced glycation end-products: comparison witn aminoguanidine // Biochem. Biophys. Res. Comm., 1996; 220: 113–119.
4. Thiamine corrects delayed replication and decreased production of lactate and advanced glycation endproducts in bovine retinal and umbilical vein endothelial cells cultured under high-glucose conditions / M. la Selva [et al.] // Diabetologia, 1996; 39: 1263–1268.
5. Thornalley P. J. The potential role of thiamine (vitamin B1) in diabetic complications // Curr. Diabetes Rev., 2005; 1: 287–298.
6. Lohmann,K.and Schuster, P., Biochem. Z., 1937, vol. 294, pp. 188–214.
7. Strumilo,S.,Acta.Biochim.Pol.,2005,vol.52,pp.759– 764.
8. Lonsdale D.— Thiamine metabolism in disease. CRC Crit Rev in Clin Lab Sci, 1975, 5, 289-313.
9. Cardoso, D.R.; Libardi, S.H.; Skibsted, L.H. Riboflavin as a photosensitizer. Effects on human health and food quality. *Food Funct.* **2012**, *3*, 487–502. [**Google Scholar**] [**CrossRef**]
10. Buehler, B.A. Vitamin B2: Riboflavin. *J. Evid. Based. Complementary Altern. Med.* **2011**, *16*, 88–90. [**Google Scholar**] [**CrossRef**]
11. Toyasaki, T. Antioxidant effect of riboflavin in enzymic lipid peroxidation. *J. Agric. Food Chem.* **1992**, *40*, 1727–1730. [**Google Scholar**] [**CrossRef**]
12. Zou, Y.; Ruan, M.; Luan, J.; Feng, X.; Chen, S.; Chu, Z. Anti-aging effect of riboflavin via endogenous antioxidant in fruit fly Drosophila melanogaster. *J. Nutr. Health Aging* **2015**, *21*, 314–319. [**Google Scholar**] [**CrossRef**] [**PubMed**]
13. Cheung, I.M.Y.; Mcghee, C.N.J.; Sherwin, T. Beneficial effect of the antioxidant riboflavin on gene expression of extracellular matirix elements, antioxidants and oxidases in keratoconic stromal cells. *Clin. Exp. Optom.* **2014**, *97*, 349–355. [**Google Scholar**] [**PubMed**]
14. Pompella, A.; Visvikis, A.; Paolicchi, A.; De Tata, V.; Casini, A.F. The changing faces of glutathione, a cellular protagonist. *Biochem. Pharmacol.* **2003**, *66*, 1499–1503. [**Google Scholar**] [**CrossRef**]
15. Altschul.R, Hoffer.A, Stephen.J.D.,1955. Influence of nicotinic acid on serum cholesterol in man. Arch.Biochem.54,558-559.
16. 89.Keener.V.S,Sanossian.N.,2008.Niacin for stroke prevention: evidence and rationale. CNC Neurosci. Ther.14, 287-294.
17. Boyonoski.A.C., Spronck.J.C.,Jacobs.R.M.,Shah.G.M.,Poirier.G.G.,Kirkland.J.B., 2002b. Pharmacological intakes of niacin increase bone marrow poly(ADP-ribose) and the latency of enthylnitrosourea-induced carcinogenesis in rats. J.Nutr. 132,115-120.
18. Birch T.W., Gyorgy P., Harris L.J. The vitamin B(2) complex. Differentiation of the antiblacktongue and the "P.-P." factors from lactoflavin and vitamin B(6) (so-called "rat pellagra" factor). Parts I-VI. Biochem. J. 1935;29:2830–2850. [PMC free article] [PubMed] [Google Scholar]
19. Per Magne Ueland , Adrian McCann, Øivind Midttun, Arve Ulvik. Inflammation, vitamin B6 and related pathways.Mol Aspects Med. 2017 №53.P.10-27. DOI: 10.1016/j.mam.2016.08.001
20. Ishihara J., Iso H., Inoue M., Iwasaki M., Okada K., Kita Y., Kokubo Y., Okayama A., Tsugane S. Intake of folate, vitamin B6 and vitamin B12 and the risk of CHD: the Japan Public Health Center-Based Prospective Study Cohort I. J. Am. Coll. Nutr. 2008;27:127–136. [PubMed] [Google Scholar]

21. Lal K.J., Dakshinamurti K., Thliveris J. The effect of vitamin B6 on the systolic blood pressure of rats in various animal models of hypertension. J. Hypertens. 1996;14:355–363. doi: 10.1097/00004872-199603000-00013. [PubMed] [CrossRef] [Google Scholar]

22. Dakshinamurti K., Dakshinamurti S. Blood pressure regulation and micronutrients. Nutr. Res. Rev. 2001;14:3–44. doi: 10.1079/095442201108729123. [PubMed] [CrossRef] [Google Scholar]

23. Okuda T., Sumiya T., Iwai N., Miyata T. Pyridoxine 5'-phosphate oxidase is a candidate gene responsible for hypertension in Dahl-S rats. Biochem. Biophys. Res. Commun. 2004;313:647–53. doi: 10.1016/j.bbrc.2003.11.149. [PubMed] [CrossRef] [Google Scholar]

24. Czeizel A.E., Puho E., Banhidy F., Acs N. Oral pyridoxine during pregnancy : potential protective effect for cardiovascular malformations. Drugs R. D. 2004;5:259–269. doi: 10.2165/00126839-200405050-00002. [PubMed] [CrossRef] [Google Scholar]

25. Merrill R.M., Taylor P., Aldana S.G. Coronary Health Improvement Project (CHIP) is associated with improved nutrient intake and decreased depression. Nutrition. 2008;24:314–321. doi: 10.1016/j.nut.2007.12.011. [PubMed] [CrossRef] [Google Scholar]

26. MacKenzie K.E., Wiltshire E.J., Gent R., Hirte C., Piotto L., Couper J.J. Folate and vitamin B6 rapidly normalize endothelial dysfunction in children with type 1 diabetes mellitus. Pediatrics. 2006;118:242–253. doi: 10.1542/peds.2005-2143. [PubMed] [CrossRef] [Google Scholar]

27. Nakamura S., Li H., Adijiang A., Pischetsrieder M., Niwa T. Pyridoxal phosphate prevents progression of diabetic nephropathy. Nephrol. Dial. Transplant. 2007;22:2165–2174. doi: 10.1093/ndt/gfm166. [PubMed] [CrossRef] [Google Scholar]

28. Graham, I. M., Daly, L. E., Refsum, H. M., Robinson, K., Brattstrom, L. E., Ueland, P. M., € et al. (1997). Plasma homocysteine as a risk factor for vascular disease. The European Concerted Action Project. Journal of the American Medical Association, 277, 1775–1781.

29. LeBlanc, J. G., de Giori, G. S., Smid, E. J., Hugenholtz, J., & Sesma, F. (2007). Folate production by lactic acid bacteria and other food-grade microorganisms. Communicating Current Research and Educational Topics and Trends in Applied Microbiology, 1, 329–339.

30. Pfeiffer, C. M., Caudill, S. P., Gunter, E. W., Osterloh, J., & Sampson, E. J. (2005). Biochemical indicators of B vitamin status in the US population after folic acid fortification:Results from the National Health and Nutrition Examination Survey 1999–2000. The American Journal of Clinical Nutrition, 82, 442–450.

31. Laurence, K. M., James, N., Miller, M. H., Tennant, G. B., & Campbell, H. (1981). Doubleblind randomised controlled trial of folate treatment before conception to prevent recurrence of neural-tube defects. British Medical Journal (Clinical Research Edition), 282, 1509–1511.

32. . Scott, J., & Weir, D. (1993). Folate/vitamin B12 inter-relationships. Essays in Biochemistry,28, 63–72

33. Seller, M. J., & Nevin, N. C. (1984). Periconceptional vitamin supplementation and the prevention of neural tube defects in south-east England and Northern Ireland. Journal of Medical Genetics, 21, 325–330.

34. Vergel, R. G., Sanchez, L. R., Heredero, B. L., Rodriguez, P. L., & Martinez, A. J.(1990). Primary prevention of neural tube defects with folic acid supplementation: Cuban experience. Prenatal Diagnosis, 10, 149–152.

35. Levine, M, Conry-Cantilena, C, Wang, Y, et al. (1996) Vitamin C pharmacokinetics in healthy volunteers: evidence for a recommended dietary allowance. Proc Natl Acad Sci U S A 93, 3704–3709.Google Scholar

36. Leggott, PJ, Robertson, PB, Rothman, DL, et al. (1986) The effect of controlled ascorbic acid depletion and supplementation on periodontal health. J Periodontol 57, 480–485.Google Scholar

37. Gale, CR, Martyn, CN, Winter, PD, et al. (1995) Vitamin C and risk of death from stroke and coronary heart disease in cohort of elderly people. BMJ 310, 1563–1566.Google Scholar