



Screening of dietary nucleotides from natural sources for therapeutic uses

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Abstract

Dietary nucleosides and nucleotides perform a range of functions in our body. They play important role in the maintenance of mitochondrial function, differentiation of hematopoietic cells, strengthen the immune system, helps in small intestine growth and development, lipid metabolism *etc.* External supplementation of dietary nucleotides is a good regimen against mitochondrial dysfunction caused by Nucleosides Reverse Transcriptase Inhibitors (NRTI) drug therapy; it has a long history of use for cancer and viral diseases *e.g.*, NucleomaxX is a dietary nucleotide supplement, and has a potential use in the treatment of mitochondrial toxicity as it contains *Mitocnol*; a sugarcane extract with a high percentage of nucleotides. Therefore, analysis of these compounds in food materials is very important for improving and assuring food quality as well as to boost Biotech Industrial sector of India; manufacturing a dietary nucleotide supplement. During the present investigation, the extraction protocol for nucleotides from different sources (sugarcane, pulses/lentils, oilseeds, yeast, mushroom and animal milks) was standardized, which is one of the simple, cost effective method for isolation of total nucleotides/nucleosides. The isolated nucleic acid was quantified using double beam UV spectrophotometer. Estimation of RNA was carried out by *Orcinol* method and DNA by *Diphenylamine* method, using nucleic acid extract obtained from different food materials. The screening of samples from different sources helped to determine the desirable concentration of nucleic acid in natural sources for dietary nucleotides. Further, investigation is in progress to study influence on animal model, developing therapeutic formulation and scale up criteria.

Key words: Dietary nucleotides, mitochondrial dysfunction, natural sources, nucleomax, sugarcane and uridine rich compounds

Introduction

The history of plants being used for medicinal purpose is probably as old as the history of mankind. Nucleotides and nucleosides are structural units in nucleic acids, co-enzymes in biological pathways as well as being sources of chemical

energy (Cosgrove, 1998). For years, nucleic acids and nucleotides were not considered essential nutrients. It was thought that the body can synthesize sufficient nucleotides to meet its physiological demands via *de novo* nucleotide synthetic pathways. For young and healthy people, getting enough nucleotides from food may be possible, but as we grow older, this pathway becomes less efficient and our ability to absorb nucleotides decreases. There are certain conditions in which the body requires dietary nucleic acids/nucleotides to meet its physiological requirements *e.g.*, during rapid growth, limited food supply and metabolic stress (Leach *et al.*, 1995; Sanchez-Pozo, 1986 and Abtahi *et al.*, 2011).

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Nucleotides are stored in a very limited quantity in our liver. Trauma, surgery, immune challenges and other stresses can rapidly deplete the body's stores of nucleotides. Nucleotides are conditionally essential nutrients involved in the small intestinal growth and development, lipid metabolism, skeletal muscle development, heart function and immune response, reviewed by Carver and Walker (1995). Nucleotides have been demonstrated to affect a number of immune functions (Pickering *et al.*, 1998; Schaller *et al.*, 2004 and 2007) including reversing malnutrition, starvation induced immunosuppression, enhancing T-cell maturation and function, enhancing natural killer cell activity, improving delayed cutaneous hypersensitivity, aiding in resistance to infectious agents and modulation T-cell responses (Kulkarni *et al.*, 1987).

Human breast milk is known to contain a significant amount of nucleotides (Leach *et al.*, 1995; Pickering *et al.*, 1998; Schlimme *et al.*, 2000 and Mateo *et al.*, 2004), which are implicated in the increased growth of the gastrointestinal tract in breast-fed infants (Gil *et al.*, 1983 and Uauy, 1989). These findings may further indicate the importance of dietary nucleotides in maintaining optimal functions of the body in certain conditions (Lea *et al.*, 2007). Furthermore, besides some free amino acids, the 5' - monophosphate nucleotides are responsible for an *umami* taste in some foods, which includes sweetness, sourness, saltiness and bitterness. These substances have become important flavor potentiators in the food industry.

An estimated 33 million people are infected with HIV worldwide. In India, approximately 2.5 million Indians are currently living with HIV. NRTIs Nucleoside Analogue Reverse Transcriptase Inhibitors (NRTIs) have a long history of use for viral and cancer diseases. Their primary mechanism of action is inhibition of DNA synthesis in target cells. They can theoretically affect every organ system through mitochondrial dysfunction (Walkar *et al.*; 2004). Mitochondrial toxicity may manifest as lipoatrophy, polyneuropathy, steatosis, steatohepatitis or acute liver failure (Setzer *et al.*, 2008). However, earlier studies indicate that the nucleotide and nucleoside, particularly uridine or uridine derivatives completely prevent and treat the mitochondrial toxicity of NRTIs by abrogating mitochondrial DNA depletion (Venhoff *et al.*, 2010; Sutinen *et al.*, 2007 and Lebrecht *et al.*, 2008).

The pharmacokinetic studies revealed that protective uridine levels could be achieved in human serum by oral mitocnol (Walker, 2003 and 2004; Banash, 2006 and Lebrecht, 2007). A food supplement containing nucleosides, NucleomaxX, has been reported to raise plasma uridine to supraphysiologic levels. It contains predominantly TAU, which has significantly greater bioavailability than pure uridine in human subjects and may be useful in the management of mitochondrial toxicity (Weinberg *et al.*, 2011).

Nutritional supplements of RNA, DNA, nucleotides and its bases are now being marketed. These products are manufactured from nucleotide rich natural sources like plant parts, mushroom, yeasts, chlorella *etc.* (Ingledeew, 1999 and Tibbets, 2002). Interestingly it is seen from the information available, that there are no reports of such products manufactured in India. In fact, the increasing numbers of AIDS and cancer patients correspondingly are increasing utility of toxic preventive drugs requiring the large-scale availability of dietary nucleotides supplements. Probably the present demand is met through imported products. Therefore, there is a need to standardize a simple and cost effective nucleotide isolation protocol from different natural sources which will be useful for mankind.

Role of nucleotides in nutrition

Benefit of nucleotides

- Promotes new skin cell growth and repair
- Neutralizes internal toxins
- Combats Mitochondrial toxicity
- Dead-stops colds and flu
- Heals wounds
- Lowers cholesterol level
- Increases energy
- Slows the aging process
- Strengthens the immune system
- Greater Muscle Mass
- Elevates Tissue Oxygenation

Source: Nucleic Acid Nutrition and Therapy.

Role of dietary nucleotides in metabolism

Nucleotides are physiological mediators in a number of metabolic processes. Nucleotides are continuously degraded and salvaged by all cells, especially in tissues with rapid turnover rates such as intestinal mucosa, skin and white or red blood cells. Cyclic adenosine and guanosine monophosphate, adenosine and Guanosine triphosphate regulate a large number of cellular events, important in regulating blood flow and smooth-muscle activity and signal transduction (Uauy, 1989).

The effects of dietary nucleotides on lipid metabolism in rats have been studied (Gil *et al.*, 1983; Uauy, 1989; Sanchez-Pozo *et al.*, 1986 and Sato *et al.*, 1995). Male rats were fed with a nucleotides-supplemented diet or a nucleotides-free diet to know the learning ability of rats fed nucleotides-supplemented diet. The result suggested that dietary nucleotides may influence lipid metabolism of the cerebral cortex and contribute to the rise in learning ability of rats (Sato *et al.*, 1995).

Dietary nucleotides and defense against infection

Various studies are available investigating the effect of supplemented nucleotides on the immune function of several species such as rodents and pigs, but also humans (Carver *et al.*, 1991).

Dietary nucleotide supplementation has been associated with both humoral and cellular immunity, but exact mechanism has been not elucidated (Kulkarni *et al.*, 1987). Dietary nucleotides play a role in the humoral antibody response to immunization of infants (Pickering *et al.*, 1998) and increased cytokine production (Carver *et al.*, 1991). Nucleotide deprivation causes a decrease in phagocytic activity, lymphokine production and inhibited lymphocyte maturation. Nucleotides modulate immune functions and are important for growth, repair and differentiation of the gastrointestinal track (Uauy, 1989). In association with dietary nucleotides, there has been shown an increase in antigen-induced lymphoproliferation, in reversal of malnutrition and starvation-induced immunosuppression (Van Buren *et al.*, 1990), as well as in natural killer cell activity and interleukin-2 production. Dietary nucleotides may block the bacterial translocation by preventing endotoxin-induced mucosal damage. Nucleotide supplementation to an infant formula reduces the counts of enterobacteria and increases the counts of bifid bacteria in the fecal microflora. These results support a low incidence of acute diarrhea in infants (Pickering *et al.*, 1998).

Dietary nucleotides in intestinal functioning and repairing

Dietary nucleotides seem to be necessary for optimal intestinal functioning and may facilitate intestinal repair (Uauy, 1994 and Sauer *et al.*, 2011). Dietary nucleotides enhance intestinal repair after injury or malnutrition (Martinez-Puig *et al.*, 2007). It would enhance growth and differentiation of the enterocytes after deprivation. It is not worthy that the nucleotide pools are low in the intestine compared with other tissue probably as a result of high activity of catabolic enzymes such as adenosine deaminase.

Dietary nucleotides reportedly promote functionality and repair in fibrotic liver. Nucleotides, the mediated precursor of nucleosides are synthesized in the intestinal lumen by epical alkaline phosphatase, and are an important component of diet during growth and tissue repair (Martinez-Puig *et al.*, 2007; Roselli *et al.*, 2007 and Shen *et al.*, 2009). In the liver, extra cellular nucleotides and nucleoside are reported to modulate hepatocytes growth and regeneration and to play an important role in the synthesis of glycogen. Orally administered nucleotides improved the hepatic function and promoted earlier restoration of nitrogen balance after liver injury or partial hepatectomy.

Dietary nucleotides against neurodegeneration.

Huntington's disease (HD) is associated with decreased activity of mitochondrial succinate dehydrogenase (complex II). *De novo* biosynthesis of uridine nucleotides is directly coupled to the respiratory chain. Cells with impaired mitochondrial function become uridine auxotrophs and can be maintained with high micromolar concentration of uridine and pyruvate. The therapeutic role of pyrimidines and possible changes in uridine content has not been assessed in neurological diseases involving mitochondrial dysfunction *in vivo*. The treatment with, uridine pro-drug 2',3',5'-tri-O-acetyluridine (PN401) was shown to be protective in the mitochondrial complex II inhibitor 3-nitropropionic acid model of Huntington's disease (Saydoff *et al.*, 2003). The higher doses of PN401 associated with optimal neuroprotection did elevate TUN to supranormal levels (Saydoff, 2006). Thus, oral PN401 treatment has neuroprotective effects in a HD model of mitochondrial dysfunction and the mechanism is more complex than correction of a pyrimidine deficit. These results suggest that PN401 may have beneficial effects in the treatment of neurodegenerative diseases such as HD (Saydoff *et al.*, 2003 and 2006).

Mitochondrial toxicity

Many drugs can alter mitochondrial functions. Clinically, this may be most relevant in the therapy or prevention of Acquired Immunodeficiency (AIDS) with nucleoside analogue reverse transcriptase inhibitors (NRTIs) (McComsey and Walker, 2004; Lewis *et al.*, 1995; Lebrecht *et al.*, 2008; Scott *et al.*, 2010 and Shoshan-Barmatz *et al.*, 2010). NRTIs were designed to inhibit an enzyme necessary for the multiplication of Human Immunodeficiency Virus (HIV). However, they also inhibit a human enzyme, called gamma-polymerase (Lewis *et al.*, 1995). Gamma-polymerase in turn, is required for the replication of mtDNA. Therefore, a copy number defect of mtDNA (called mtDNA-depletion) can result from the prolonged exposure of HIV-patient with these antiviral agents. As a consequence of mtDNA-depletion, the mtDNA-encoded respiratory chain subunits cannot be synthesized in adequate amounts and respiratory chain function fails (Lewis *et al.*, 1995 and 2003). Mitochondria are in every cell of our body, mitochondrial toxicity can theoretically affects every organ system (Figure 1.).

Mitochondrial toxicity may manifest as lipatrophy, polyneuropathy, steatosis, steatohepatitis or acute liver failure. Probably the most stigmatizing affect of NRTI-related mitochondrial toxicity is lipatrophy. Lipatrophy consist of a irreversible loss of subcutaneous adipose tissue, which occurs mainly in the face, but also affects the buttocks, abdominal area and the extremities (Carr *et al.*, 2000).

Mitochondrial toxicity to the liver results in elevated liver enzymes, and probably also increased generation of lactate.

Anemia and leucopenia are side effects of zalcitabine (ddC), didanosine (ddI), stavudine (d4T) and Zidovudine (AZT). Zidovudine is a weak inhibitor of gamma-polymerase. However, another mechanism of zidovudine might cause mtDNA depletion independent from gamma-polymerase inhibition. AZT is an inhibitor of mitochondrial thymidine kinase type 2 (TK2) and as such, interferes with the synthesis of natural pyrimidine nucleotides, thus, potentially impairing the formation of mtDNA. Indeed, inborn defects of TK2 are known to cause mtDNA depletion in human muscle tissue (Saada, 2001). It has also been demonstrated that AZT can be non-enzymatically converted into d4T within the body, at least within some cells, (Becher *et al.*, 2003 and Bonora *et al.*, 2004). They are probably a consequence of mitochondrial toxicity to the heart and skeletal muscles results in weakness during exercise. Prolonged exposure of HIV-patients to some NRTIs damages the peripheral nerves. Fancosis syndrome and phosphate loss in the kidney can be a consequence of mitochondrial toxicity to the tubules in the kidney. The examples of Nucleoside-analogue reverse transcriptase inhibitors (NRTIs) tabulated in Table 1.

Uridine in the reduction of mitochondrial toxicity

Uridine is synthesized by the power plant of the human cell known as the mitochondria. Uridine is also required for many other metabolic pathways (Connolly and Duley, 1999). Humans are normally able to produce uridine, but the ability to do so requires intact mitochondria. Numerous data indicated that uridine (in concentration of 50 to 200mM) completely prevents and treats the mitochondrial toxicity of pyrimidine nucleoside analogue by abrogating mtDNA depletion or in the case of zidovudine through an unknown mechanism (Setzer *et al.*, 2008). Indeed, uridine abolishes all the effects of mtDNA depletion in hepatocytes and normalizes lactate production, cell proliferation, the rate of cell death and intracellular steatosis in animal model as well as HIV-positive patients (Walker *et al.*, 2003 and 2004; Banasch *et al.*, 2006 and Lebrecht *et al.*, 2007). The mechanism of action of dietary nucleotides shown in Figure 2.

New data indicate that uridine is also able to prevent ddC-induced hepatotoxicity (Lebrecht *et al.*, 2007) and AZT-induced myopathy (Lebrecht *et al.*, 2008), cardiomyopathy and neuropathy (Balcarek *et al.*, 2010) and in mice (Venhoff *et al.*, 2010). Clinical trials indicate that uridine can be safely administered to humans even in high doses (Kelsen *et al.*, 1997 and Van Groeningen *et al.*, 1986). There are no known negative interactions of uridine with antiretroviral treatment (Koch *et al.*, 2003; McComsey *et al.*, 2004 and Sutinen *et al.*, 2007). The lack of effective treatment modalities for NRTI-induced toxicity has been a very frustrating issue in the HIV metabolic field. Increasing uridine may be promising

in the treatment of some symptomatic or life threatening mitochondrial toxicities in HIV- infected patients. Uridine is available commercially as a dietary supplement NucleomaxX (www.nucleomaxx.com and www.mitocnol.com).

Dietary nucleotide supplements

Nucleotides are naturally present in all foods and feeds of animal and vegetable origin (Mateo *et al.*, 2004). The mother's milk is a rich source of nucleic acids, especially RNA and nucleotides. Dietary sources are found to varying degrees in many foods like lamb liver, mushrooms (but not fruit and other vegetables) all rich in nucleotides. A dietary source of RNA includes seafood (Danfegan, 2004), nuts, beans, mushrooms, beef, broth and vegetable soups (Tibbets, 2002). RNA and DNA nutritional supplements are available in the form of liquid or tablets. There are a few medical foods and eternal supplements containing RNA and nucleotides.

Neuroceutical product IntestaidIB

IntestAidIB, which are being sold in the form of capsules, are carbohydrate based and dosage consists of one 500 mg capsule three times a day with meals. RNA and the specific purified nucleotides are the extracts of yeast.

Cell food DNA/RNA (formerly MethusaLife)

Cell food DNA/RNA only contains the individual bases of DNA and RNA for safety and a high potency of absorption and utilization. Cell food DNA/RNA can be a very powerful tonic to sustain and boost the functions of the most vital glands in the body. These glands set our level of energy, our ability to respond to stress, maintain strong immune defenses, prevent dehydration and balances hormone which is essential to a high quality of life. It also increases long term memory and longevity.

Quantum-RX

A proprietary formula of nucleotide concentrate (highly bio-available nano-protein RNA and DNA factors) with their essential phyto-nutrient synergists and co-factors promotes rapid cellular rejuvenation, increased vitality, healthy immune system, protein support and DNA repair. The Quantum Nutrition Effect: When premier nutrients are combined together, their effect is far greater than the sum of their individual benefits by a factor of 2 to 100fold or more.

NucleomaxX

NucleomaxX is a food supplement of potential use in treatment of mitochondrial toxicity, which is developed to combat side effects of HIV medication, (Walker *et al.*, 2003 and 2004; Banasch *et al.*, 2006 and Lebrecht *et al.*, 2007). One sachet contains 6g nucleosides from extract of sugar cane (Mitocnol). Ingredients include extract from sugarcane (67%) with high content of nucleosides (17% of uridine), milk protein, aroma and sweeteners. An intake of three sachets per day for free consecutive days every month is recommended.

The cost of 9 sachet of NucleomaxX, not including shipping and taxes, is approximately: \$133, (www.nucleomaxx.com). Moreover long term uridine supplementation used in a rare genetic disease (hereditary orotic aciduria), appears to be safe.

Sugarcane a value added product

Sugarcane juice is considered nutritious as it contains natural sugars, minerals and organic acids. It has many medicinal properties and is used in ayurvedic preparations. It is great for recharging energy because it contains rich carbohydrate and iron. It is a diuretic, expectorant, laxative, haemostatic, and a cardio-tonic. It helps in general debility and is believed to be an aphrodisiac. Sugarcane juice keeps the urinary flow clear and helps the kidneys to perform their functions properly. It is now clear that the sugarcane has many medicinal values (www.mitocnol.com). Therefore, the present research work has an objective to know why some commercial food supplements use sugarcane extract as their main ingredient. It is also noticed by many articles that Uridine, and 2', 3', 5'-tri-O-acetyluridine (TAU) nucleosides were the only nucleosides present in analytically significant quantities in NucleomaxX product (Weinberg *et al.*, 2011). It may be the genetic content of sugarcane which is responsible for producing more amount of Uridine compared to other plant species. NucleomaxX is quite expensive and a three month course can cost upwards of \$450. An attempt was made to minimize the cost of nucleic acid isolation protocol from sugarcane with the help of standardizing simple nucleic acid isolation protocol.

Materials and Methods

Experimental sample

Sugarcane samples were collected from Gulbarga and North Karnataka region, which includes sugarcane leaves, juice, molasses and bagasse. Similarly the husk of pulses/lentils [*Cajanus Cajan*, (peagen pea); *Phaseolus aureus*, (green gram); *Lens esculenta*, (Masoor); *Phaseolus spp.* (white beans, black beans and Kidney beans)] and Oilseeds of [*Glycin max*, (soybean); *Arachis hypogaea*, (Ground nut); *Helianthus annuus*, (Sunflower), *Sesamum indicum* (sesame)] and cereals were collected. The other samples used for present investigation include milk, yeast and mushroom.

Analytical procedure

The extraction protocol for nucleotides was standardized with necessary modification, as according to Perin *et al.* (2001). Three months old sugarcane young leaves and upper young portion of stem, its juice, molasses and bagasse were taken as well as seeds of pulses/lentils were also used for estimation of dietary nucleotides. Water soluble samples were suspended in water at 1 g /10 mL of final volume. They were then stirred

at for 15 min before an aliquot was removed for perchloric acid treatment. Then, equal amount of ice-cold 13% perchloric acid was added and mixed. The mixture was allowed to stand on ice for 10 min and then centrifuged on a bench-top centrifuge at 5000 rpm for 20 min at 4°C. An aliquot of the supernatant were introduced into a beaker and the pH adjusted to 4.0 with 5M KOH. Then the samples were diluted with water and left in ice bath for at least 1 h to precipitate all the potassium perchlorate, then the sample was centrifuged to remove the pellet of KClO₄. The supernatant was filtered through a 0.45 mM membrane filter before analysis.

Analysis of DNA and RNA

Estimation of RNA by orcinol method

In a set of test tubes 0 to 1 ml of stock solution of standard RNA was taken and the final volume was made up to 1ml with distilled water, 4 ml of the Orcinol reagent was added and vortexes. The test tubes were covered with aluminum foil, secured with a rubber band and placed in boiling water for 15 minutes; tubes were cooled in a running tap water to room temperature. Absorbance was measured at 665 nm against Orcinol reagent as a blank. A standard graph was drawn by plotting the values as a function of RNA concentration (Mohammed Kamali and Hamid Manhour, 1969).

Estimation of DNA by diphenyl amine method, (DPA)

Stock solution of standard DNA was taken in clean test tubes up to 0 to 1 ml and the volume was made up to 1 ml by adding distilled water. 5 ml of diphenyl amine reagent was added to each test tube and vortexed. Sample containing tubes were placed in a boiling water bath for 10 minutes, and cooled in room temperature; the absorbance was noted at 595 nm. A standard graph was drawn by using the values as a function of DNA concentration (Burton, 1985).

Double beam UV spectroscopy analysis of nucleic acid derivatives of different sources

The double beam UV spectra analysis of different nucleic acid extracts were analyzed at wavelength range of 200-400 nm. These samples were analyzed against distilled water as blank. The max spectral absorption was recorded and compared with standard (Cherry, 1992).

Results and Discussion

Extraction of nucleotide by perrin method

The present investigation has been attempted to know the nucleic acid content from plant material with non toxic inexpensive method, suitable for therapeutic application. The extraction protocol for nucleotides was standardized with necessary modification, according to Perin *et al.* (2001).

Initially the standardized protocol for isolation of DNA was used, which is suitable for sugarcane to avoid the interference

by the carbohydrate and phenolics which are major constraints in solution of DNA from the plant material (Virupakshi and Naik, 2007). Later a simple non toxic salt based nucleic acid isolation protocol was used to avoid expensive and hazardous chemicals like phenols, chloroform, EDTA, sodium acetate and tris base, method modified by Aljanabi and Martinez (1997). These two methods were restricted only for DNA isolation. In the first step of research, during screening of natural sources for their high nucleic acid content, it was found that Perrin *et al.* (2001) is a simple and easiest method as compared to others. This protocol utilizes a numbers of steps to extract nucleic acid (DNA/RNA) from the different samples so that time consumed is minimized. The extracted solution was used for estimation of DNA and RNA using diphenyl amine and orcinol method (Burton, 1985). The same method can be used to study various parameters of these dietary nucleotides and their derivatives efficiently in natural sources by using different chromatography methods such as; RP-HPLC, IELC, CE, HPLC/Diode array, HPLC-MS-MS and LC-MS/MS (Inoue *et al.*, 2008; Vinas *et al.*, 2009; Tsopmo and Muir, 2010 and Yiping *et al.* 2011).

Estimation of nucleic acid

Among the various colorimetric methods for RNA and DNA estimation, the Orcinol and DPA reaction is the most sensitive and commonly employed technique for purine-bound ribose and deoxyribose quantification (Mohammed Kamali and Hamid Manhouri, 1969 and Burton, 1985). The screening of these samples from different sources helped to determine the concentration of nucleic acid in natural sources for dietary nucleotides, which is shown in Table 2. The desirable

concentration of nucleic acid was observed in *Saccharum officinarum*, *Glycin max*, *Phaseolus vulgaris*, *Phaseolus aureus*, *Phaseolus mungo*, *Saccharomyces Crevisiae*. The sugarcane extracts were having maximum concentration of DNA as well as RNA as compared to other sources. Soybeans, white beans, yeast and goat milk has high concentration of RNA. Lowest concentration of nucleotides was observed in mushroom. Result represented in Figure 3.

Double beam spectroscopy

Spectroscopy is used for quantification of nucleic acids showing a strong absorbance in the region of 240 to 270 nm. Purine and pyrimidine ring system can be protonated and, therefore, the spectra of DNA and RNA are sensitive to pH. The detection and quantification of nucleic acid by UV-spectroscopic method routinely performed and became a valuable tool for analysis of DNA, RNA and related molecules (Cherry, 1992). The polymetric DNA and RNA show a broad and strong absorbance near to 260nm. At this wavelength, absorbance is proportional to nucleic acid concentration. This relationship is so well characterized that ultra violet absorption is used to accurately determine the concentration of nucleic acid in solution. The relationship between DNA concentration and absorbance is linear. In a spectral analysis at acidic pH, the maximum absorbance was recorded at the wavelength of 280 to 300 nm. Sugarcane, soybean, bean seeds, yeast and milk showed maximum absorption compared with standard, the results represented in Table 3. and Figure 4. This data is helpful for screening and selection of plant material which is rich in nucleotide concentration.

Table 1. Examples of nucleoside-analogue reverse transcriptase inhibitors (NRTIs)

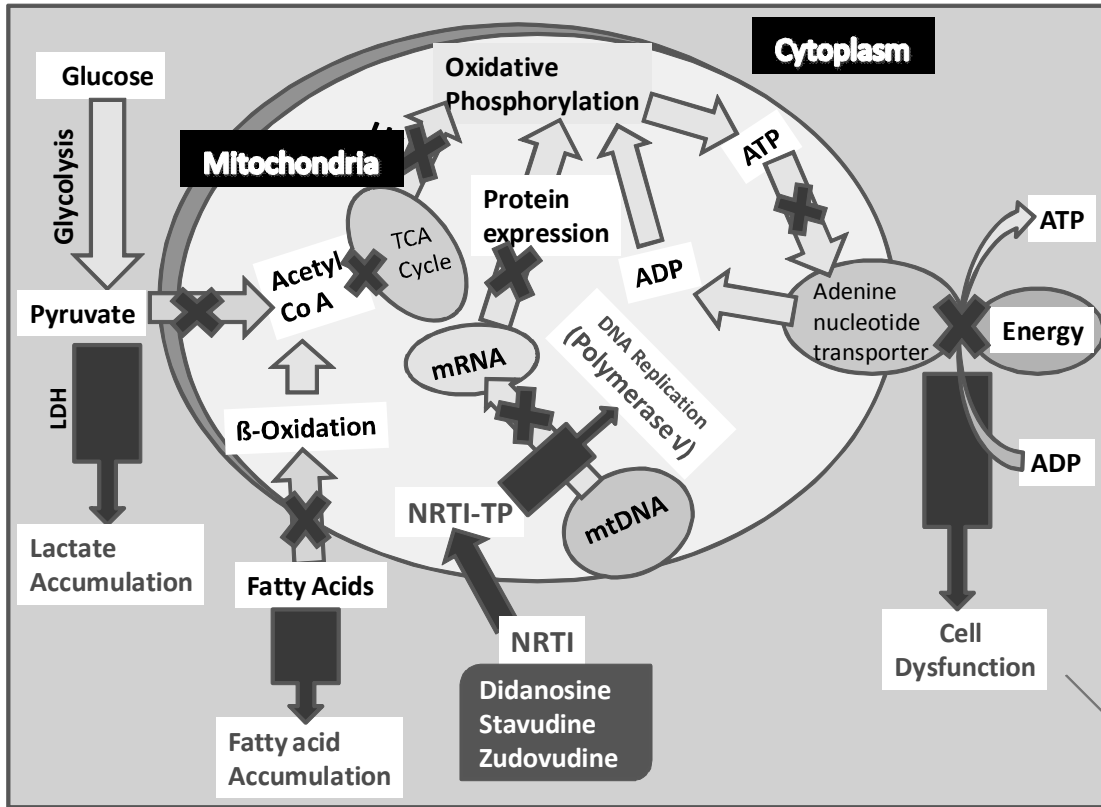
Trade Name	Generic Name	Abbreviation
Epivir	Lamivudine	3TC
Combivir	Lamivudine + Zidovudine	3TC+AZT
EasyTab	Lamivudine + Abacavir	3TC+ABC
Emtriva/Covirazil	Emtricitabine	FTC
Hivid	Zalcitabine	DDC
Retrovir	Zidovudine	AZT
Trizivir	Lamivudine + Zidovudine + Abacavir	3TC+AZT+ABC
Viread	Tenofovir	TNF
Zerit	Stavudine	d4T

Table 2. Estimation of RNA and DNA

Sl. No.	Samples	RNA (mg/ml)	DNA (mg/ml)
1	Sugarcane extract A	0.11	0.02
2	Sugarcane extract B	7	0.15
3	Sugarcane extract C	9	0.3
4	Sugarcane extract D	20	4
5	Sugarcane extract E	17	5
6	Mushroom	2	0.1
7	Yeast	9.4	0.1
8	Milk	8	0.8
9	White beans	9	4.5
10	Soybean	5.3	0.52
11	Black beans	5	0.62
12	Kidneybeans	2.6	0.22
13	Ground nut	8	0.8
14	Red gram	3.8	0.5
15	Green gram	4.4	0.4
16	Black gram	3.9	0.22
17	Lentils	3.6	0.25
18	Sorghum	2	0.14
19	Wheat	2.2	0.12

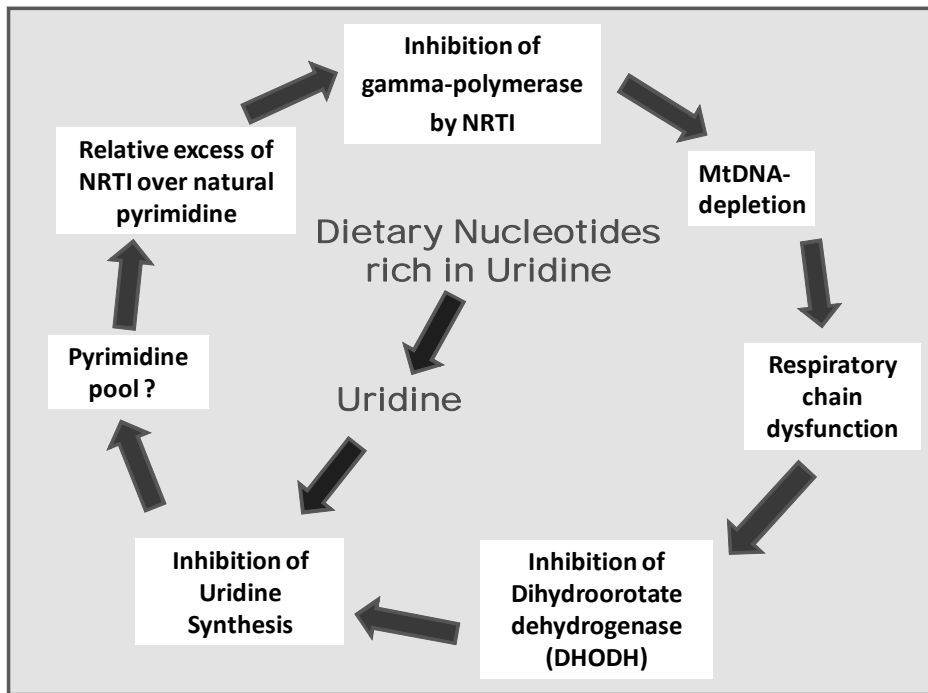
Table 3. Estimation of nucleotides by double beam spectroscopy

Samples	Max Absorbion (nm)	Optical Density
Milk	284	3.666
Stand. Uridine	286	2.96
Mushroom	288	0.998
White beans	290	4.452
Soybeans	292	3.066
Yeast	292	4.515
Sugarcane extract E	294	7.655
Sugarcane extract D	298	9.993



G.R.N.

Figure 1. Mitochondrial dysfunction with NRTIs



G.R.N.

Figure 2. Mechanism of action of dietary nucleotide

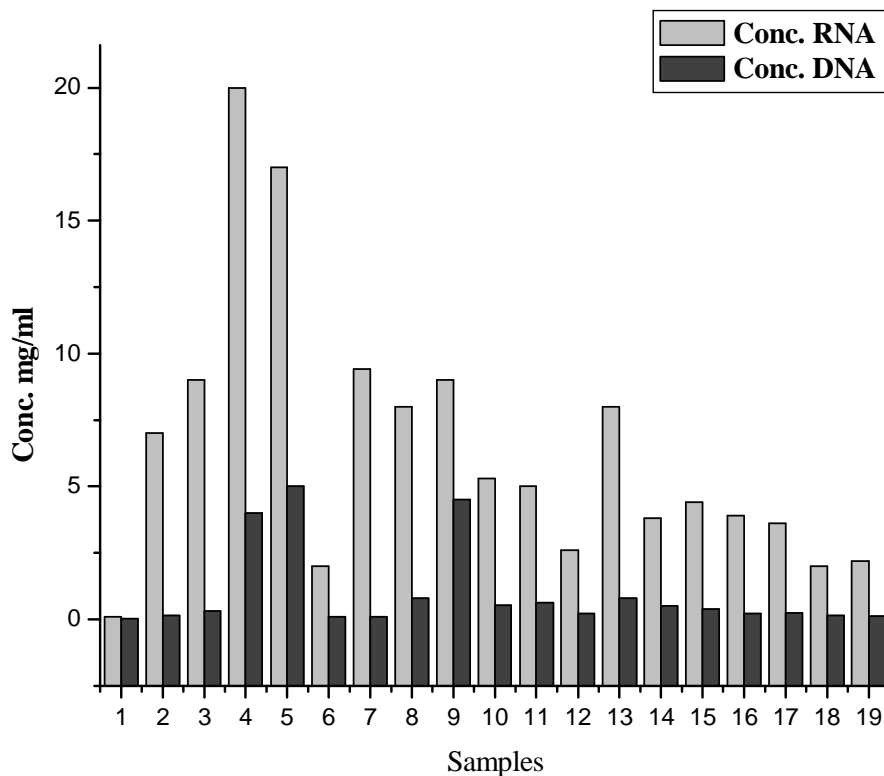


Figure 3. Estimation of RNA by Orcinol method and DNA by Diphenyl amine method

1. Sugarcane extract A; 2. Sugarcane extract B; 3. Sugarcane extract C; 4. Sugarcane extract D; 5. Sugarcane extract E; 6. Mushroom; 7. Yeast; 8. Milk; 9. White beans; 10. Soybeans; 11. Black beans; 12. Kidney beans; 13. Ground nut; 14. Red gram; 15. Green gram; 16. Black gram; 17. Lentils; 18. Sorghum; 19. Wheat.

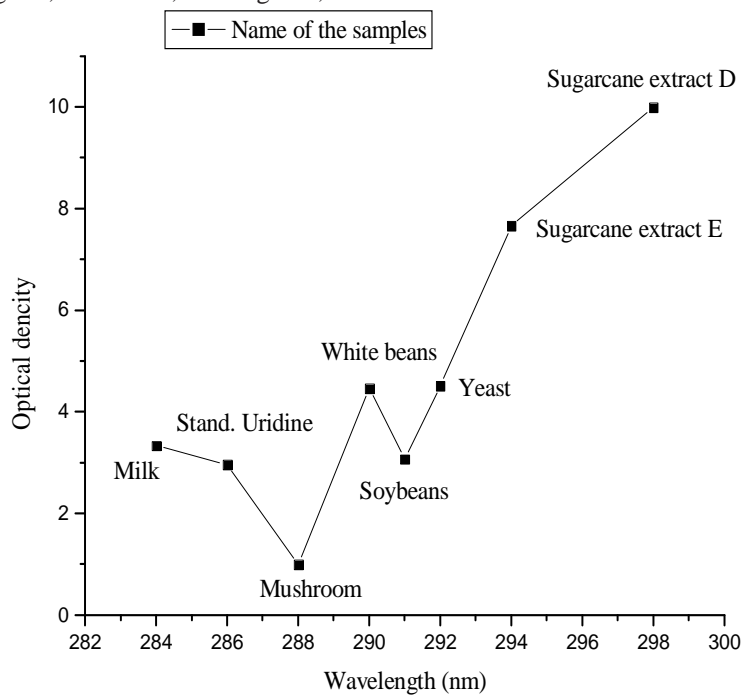


Figure 4. Double beam spectroscopy analysis of different dietary nucleotide sources

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