May 2017
Issue 1

Biochem *Trends*

The newsletter of the College of Biochemists Sri Lanka

Controversy: Glyphosate Ban

Is there adequate scientific evidence for toxicity of Glyphosate? Senior Professor Deepal Mathew questions the decision for the import ban of glyphosate in Sri Lanka.

Page 3

Erythrocyte Antioxidant Status in Development of Cataract

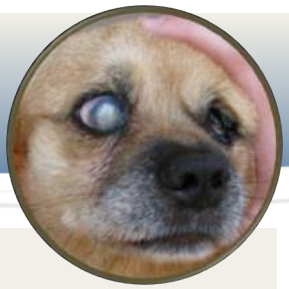
Hemantha Peiris PhD (Qld, Australia) ACBI
Professor of Biochemistry
Faculty of Medical Sciences,
University of Sri Jayawardenapura

A free radical is an atom that contains an unpaired electron and this configuration renders free radicals highly unstable and chemically reactive. There is strong evidence for roles of free radicals in a wide variety of diseases and degenerative conditions including pathogenesis of atherosclerosis, cancer and cataract. Oxidative stress may result when the cellular antioxidant defense mechanisms are unable to keep pace with the detoxification of reactive oxygen intermediates (ROI). ROI mediated peroxidation of membrane lipids can cause extensive damage to proteins, leading to irreversible deleterious effects.

Continued on Page 4

Inside this issue

CBSL hosted the FAOBMB Executive Committee meeting in June 2016, in Sri Lanka.

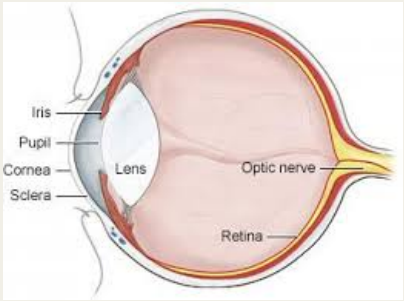


Editorial

Silent Enemies, Fallen Heroes

As silently as the gathering darkness envelops the evening shadows at dusk, the developing cataract clouds the lens of the eyes, impairing vision in the evening of life. The leading article in this issue of Biochem Trends discusses the mechanistic aspects of the development of cataracts, and potential biomarkers that identify individuals at risk.

Cells in our body are constantly exposed to oxidative stress from reactive oxygen species and free radicals. In response, a reservoir of antioxidants maintains a redox homeostasis, in order to prevent damage to cellular components. Glutathione in its reduced form (GSH), is considered to be the most important antioxidant molecule in the lens, and is required for removal of H_2O_2 and lipid hydroperoxides. The synthesis and recycling of reduced glutathione diminishes progressively with age. The Hexose mono-phosphate (HMP) shunt pathway metabolizes glucose, where it plays a vital role in providing the required NADPH for the reduction of oxidized glutathione, catalyzed by glutathione reductase. In Diabetics, excess glucose may stimulate the Polyol pathway via aldose reductase, which leads to the formation of sorbitol from glucose. Accumulation of sorbitol, which causes osmotic stress in the lens is believed to play a role in initiation of diabetic cataract.



Prof. Peries's research studies may indicate that the antioxidant status in erythrocytes may reflect the status in the lens, as it has been found to be associated with the development of cataract.

Continued on page 7

Message from the President, CBSL

One of the main objectives in setting up of the College of Biochemists was to disseminate new insights into biochemistry to the general public. Biochemistry plays a major part in our day-to-day life and is associated with both good health and disease. As a new organization getting information to the public is not an easy matter. Thus I am sure that the newsletter of the College of Biochemists will be the initial step in familiarizing Biochemistry among the public and it will also provide an opportunity to the scientific community to express their views.

The editorial staff has worked hard to produce this newsletter and their tireless efforts have borne fruit. Thus I take this opportunity commend their dedication and hard work and wish them all the success in their future endeavors.

Happy reading

Dr. P. P. Rasika Perera

Prof. Majid stresses a point…

CBSL received the **FAOBMB Travel Lectureship award** to host Prof. Majid Ghayour-Mobarhan (MD, PhD), Director of Department of New Sciences and Technology & founding Member of Cardiovascular Research Center, Mashad University of Medical Sciences, Iran in Sri Lanka.

Prof Majid, presented his research at the inaugural symposium of the CBSL and gave a series of lectures in the Colombo Medical Faculty and Sri Jayawardenapura Medical Faculty. He was also invited by Nutrition society of Sri Lanka and Sri Lanka Association of Young Scientists (SLAYS) to give a motivational talk.

Glyphosate ban: Is it a scientific decision?

CPDW Mathew PhD
Senior Professor
Dept. of Biochemistry and Molecular Biology
Faculty of Medicine, University of Colombo
Past president, Sri Lanka Association for the Advancement of Science (SLAAS)

Glyphosate is a derivative of the amino acid glycine. It is a broad-spectrum systemic herbicide that has been used since 1970s and is the most used herbicide in the world. In the United States, 180 million pounds are used annually for crop protection and another 5 to 8 million pounds for domestic purposes. The mode of action of glyphosate is to inhibit the shikimate pathway used by plants and many bacteria to produce aromatic amino acids. The enzyme inhibited is 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). Glyphosate inhibits the enzyme by binding to the active site. As animals do not have the shikimate pathway, it has very low toxicity to animals.

Glyphosate is used in 130 countries. In September 2013 El Salvador banned glyphosate use after 2015. In 2014 Netherlands banned the use of glyphosate for domestic use. In 2015 in France, it was advised to sell glyphosate from locked cabinets. Sri Lanka and Bermuda banned the use of glyphosate in 2015. It is very obvious that developed countries do not consider that the commercial use of glyphosate is harmful to humans.

Toxicity of glyphosate

All chemicals are toxic. LD_{50} is used as the standard measure for toxicity of chemicals. LD_{50} indicates the quantity of the chemical that is required to kill 50% of an experimental rat population. This is expressed as mg/kg body weight. Therefore there is a range for the toxicity of chemicals. All chemicals are categorized as (i) Very Toxic (LD_{50} less than 25mg/kg) (ii) Toxic (LD_{50} 25-200mg/kg) or (iii) Harmful (LD50 200 to 2000mg/kg).

Glyphosate has a LD_{50} of 5000mg. The LD_{50} of some common chemicals are; sodium chloride 3000mg/kg, ethanol 7000mg/kg.

A systemic review conducted in 2013 by the German Institute of Risk Assessment that examined epidemiological studies, animal studies, and *in vitro* studies concluded that no classification and labeling of carcinogenicity is warranted for glyphosate and did not recommend a carcinogenicity of either category 1A or 1B. The International Agency for Research on Cancer (IARC) has classified glyphosate as probably carcinogenic in humans (category 2A). However, it must be noted that IARC does not conduct risk assessment and they classify carcinogenic *potential*. This means a few positive findings are sufficient to declare a hazard although negative studies are also present.

Fate of Glyphosate in the Environment

As Glyphosate has a charged phosphate group, it binds strongly to soil. Therefore water pollution is very low. Glyphosate is degraded to aminomethylprosphonic acid (AMPA) and carbon dioxide by soil microbes. The half-life of glyphosate varies from 2 to 197 days. Glyphosate and its degraded product AMPA are considered to be much less toxic than herbicides that were used prior to glyphosate.

Glyphosate and Genetically Modified Crops

Some microorganisms have the enzyme 5-enolpyruvylshikimate-3-phosphate syntase, which is resistant to glyphosate inhibition. An enzyme that is resistant to glyphosate and that has sufficient activity to continue plant growth was discovered from *Agrobacterium sp.* The gene was cloned and transferred to soya bean in 1996. Glyphosate resistant soya bean has been commercialized. Other genetically modified crops that are resistant to glyphosate are maize (corn) and cotton. In 2010, in the United States, 70% corn, 78% cotton and 95% soya bean planted were genetically modified plants that were herbicide resistant.

Continued on page 7

Continued from page 1

Under physiological conditions, erythrocytes serve an important function as circulating scavengers of reactive oxygen species (ROS). Erythrocytes are well equipped with non-enzymatic antioxidants such as glutathione, ascorbic acid and vitamin E. Furthermore, compared to cells in other tissues of the body, erythrocytes exhibit high activities of important antioxidant enzymes including glutathione peroxidase (GPX), superoxide dismutase (SOD), catalase and glutathione reductase. It has been shown that erythrocytes significantly contribute to detoxify ROS and thus protect from intense oxidative stress. In this context, the erythrocyte antioxidant model was studied with respect to oxidative stress in development of cataract.

The health of the lens depends greatly on the reducing state of proteins in the lens. Cataract develops due to a) concentrated action of accumulated sorbitol within the lens and b) the oxidized state of the lens proteins (crystallins) in diabetics (osmotic cataract). The biochemical events leading to the formation of cataract in diabetic and non-diabetic subjects can be separated into two hypothetical phases (Phase I and Phase II). The phase 1 predominates in uncontrolled diabetes, where the km value of hexokinase is exceeded by high glucose levels, thus producing high levels of sorbitol via aldose reductase in a reaction utilizing NADPH, which is produced by the HMP shunt via G6PD. Any deficiency of G6PD or NADPH would therefore slow down accumulation of sorbitol and will delay cataract formation. If there is adequate NADPH supply this would augment the formation of diabetic cataract. However in such patients, if there is high demand on the reduced glutathione (GSH) levels due to increased oxidative stress, it would create a higher demand on the common NADPH source. Therefore the concerted action of Phase I and Phase II will predispose these diabetic patients to a higher risk of cataract formation.

In the case of non-diabetic patients, the cataract formation could be explained mainly by events in Phase II. For example, in a non-diabetic senile cataract a high oxidative stress could be due to increased ROI production or UV radiation, which will impose a high demand on the reduced glutathione (GSH). If in such subjects, this capacity to maintain adequate GSH levels is diminished by inadequate NADPH levels (due to low G6PD) they would be predisposed to cataract formation. Unlike in the previous case there is no involvement of Phase I and the events are restricted to phase II only, but the common denominator is the supply of NADPH for GSH. The results of our previous published research pertaining to cataract, is explained on the basis of this hypothesis.

Highlights from the inaugural CBSL symposium

Continued….

Research findings revealed that the GSH levels of all subjects with cataract were significantly lower than that of non-cataract subjects. In high oxidative stress there will be an increased drain on the GSH pool leading to increased replenishment of GSH via NADPH from the HMP pathway. However, if the drain on the NADPH is higher due to diabetes mellitus, these subjects will show higher risk of development of cataract than the normal. In senile cataract, the decline of G6PD activity may be a consequence of increased exposure to environmental factors such as ROI, and UV radiation. A high content of GSH in the lens is believed to protect thiols in structural proteins and enzymes, keeping them in the reduced state for proper biological functions. Decreased GSH levels were found to be associated with senile cataracts when compared to the control subjects Thus, high levels of GSH would have aided the prevention of development of cataract in these subjects. This prevention must have been supported by adequate levels of G6PD resulting adequate supply of NADPH preventing the formation of cataract. Comparisons between non-diabetic senile cataract and diabetic cataract (osmotic cataract) subjects indicated significant decrease in catalase, glutathione peroxidase (GPX) and superoxide dismutase (SOD) activities in non-diabetic cataract group which showed that diabetic cataract is associated with higher levels of GSH (reduced glutathione) and low levels of lipid peroxidation in the erythrocytes as opposed to senile cataract, indicating a non-significant role for GSH in the pathogenesis of diabetic cataract.

It is suggested that a decrease in the antioxidant status of the erythrocytes may increase the oxidative damage in tissues, including oxidative modification of lens proteins observed in cataract. The chronic oxidative stress generated by the polyol pathway is likely to be an important contributory factor in the slow and progressive development of diabetic cataract. In addition, our studies also revealed that the erythrocyte antioxidant enzyme activities, mainly catalases, GPX and SOD are decreased in senile cataract as opposed to osmotic cataract.

In summary, erythrocyte antioxidant enzymes, G6PD, GSH, catalase, GPX, SOD and lipid peroxidation rate are sensitive indicators for the changes taking place in pathogenesis of cataract. Furthermore, individuals with G6PD deficiency are prone to develop cataract and if G6PD deficient patients are diabetic they are more vulnerable to cataract formation. Hence, these findings indicate the usefulness of RBC antioxidant enzymes as markers to identify persons at risk of developing cataract.

CBSL symposium & cultural visit to Dalada Maligawa

CBSL Buzz

As one should expect, CBSL doesn't just do things…. it does things spectacularly! CBSL held as its first main activity, an international symposium on the 22nd of June 2016, in collaboration with the Federation of Asia Oceanian Biochemists and Molecular Biologists (FAOBMB). The event, which was attended by ~100 participants was graced by a glittering panel of international speakers from Japan, China, Australia, Malaysia etc who are members of the Executive Committee of the FAOBMB. In addition to giving us a glimpse of their research, other topics of discussion were balancing academic work and family as well as fellowships obtainable through the FAOBMB. Our young biochemists also got a chance to interact and discuss their work with these eminent scientists on a more informal level during breaks.

CBSL, which is the representative constituent member of the FAOBMB from Sri Lanka, also hosted the FAOBMB Executive Committee meeting in Sri Lanka. Several of our council members had the privilege of sitting in as observers at this meeting, getting a glimpse into the running of international scientific committees, an important learning experience for the young and active CBSL council members.

The EC committee was so impressed and charmed with CBSL (and of course Sri Lankan hospitality!), that they have invited us to host the 28th FAOBMB Conference in 2020 in Sri Lanka!

CBSL has already taken great strides in promoting disseminating Biochemistry research and training, and will continue its endeavor in the future. This is an invitation for all biochemists (and Molecular Biologists of course) to join, contribute to our effort and most of all, be a part of the adventure!

Editorial…. continued from page 3

Glyphosate was once considered the hero of the agricultural world. Discovered as a potent systemic herbicide and patented as 'Roundup' by the multinational corporation Monsanto, glyphosate formulations became one of the most utilized herbicides worldwide. However, in recent years, fears of glyphosate-mediated health issues, including CKDu, have become a topic of concern.

In 2015, the import of glyphosate was banned in Sri Lanka, leaving farmers distraught, with no suitable alternative.

Arguments for the safety of glyphosate is essentially based on the fact that it inhibits a metabolic pathway that is absent in humans, and therefore could have no effect on humans. On this basis, Prof Mathew debates the decision to ban glyphosate in Sri Lanka.

However, it is important to give due consideration to the effect of glyphosate on the myriad microorganisms that dwell in our bodies and are now known to serve important regulatory functions pertaining to health. Further, studies carried out using purified glyphosate may not reflect the effect of the actual glyphosate formulations, a fact that is becoming apparent in recent studies. Studies using cell lines as well as small animal models have shown that certain glyphosate formulations do have toxic effects, including oxidative stress.

It is however, unclear as to whether the decision to ban glyphosate import into Sri Lanka was based on due consideration and assessment of scientific investigations. However, the future may yet reveal the wisdom of the decision.

Sharmila Jayasena PhD.
Editor

Members may submit academic or opinion articles for publication in future issues of Biochem *Trends*. Please contact editor at sharmjay777@gmail.com

Glyphosate Ban… continued from page 3

Cost of glyphosate ban to Sri Lankan economy

In Sri Lanka, a major component of the cost of production of agriculture is weed control. It is vital in major crops like tea, paddy and coconut. The price of production of a kilogram of tea increased from Rs. 126 in 2000 to Rs 422 in 2013. Cost of production of paddy in the Polonnaruwa district increased from Rs. 15.70 per Kg in 2005 to Rs 32.49 in 2013. The production cost for 1000 coconuts in 2002 was Rs. 4154.00 and in 2013 it had increased to Rs. 12684.00. The use of manual labour for weeding may lead to a possible collapse of the plantation industry. The use of alternative herbicides will not only be more expensive, it will also lead to more serious environmental damage.

Chronic Kidney Disease of Uncertain Etiology (CKDu)

Chronic Kidney Diseases of Uncertain Etiology (CKDu) is prevalent in the North Central province. Studies by Jayathilaka co-workers indicate that the risk is higher in males above 39 years. Further, farmers engaged in Chena cultivation were more susceptible than those involved in paddy cultivation. They observed that food items in the endemic areas contained cadmium at a higher level than the reference level and concluded that cadmium exposure was a risk factor. Genetic susceptibility studies also indicate that some individuals may be predisposed towards the development CKDu on exposure to risk factors.

Chronic Kidney Diseases in Other Countries

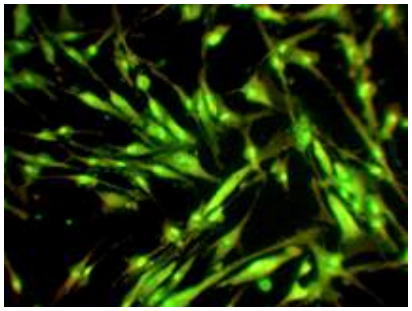
Danubian Endemic Familial Nephropathy (DEFN) was first identified in the 1920s. DEFN was observed in small discrete communities along the Danube River. This is only observed in adults. The disease is observed in people who are resident in endemic areas for more than 15 years. Over the years more than 25000 cases have been reported. The first hypothesis was intoxication with heavy metals. The etiology of DEFN is still uncertain. However chronic exposure to dietary aristolochic acid has been identified as a major risk factor in 2011. Aristolochic acid is present in seeds of *Aristolochia clematitis* (European birthwort), an endemic plant in the effected region. The seeds are mixed with wheat used for producing bread. Aristolochic acid present in Chinese herbal medicine is associated with a related condition known as Chinese herbs nephropathy.

In hot low land Central America a mysterious kidney disease (CKDu) has affected persons working in sugar cane plantations. It was first assumed to be caused by infectious diseases and heat stress. Later it was blamed on herbicide and insecticides. However it was discovered that in El Salvador CKDu does not affect people working in regions higher than 500 meters above sea level. Pesticide expose is the same in both populations. Therefore heat stress and chronic dehydration is assumed to play a major role in the development of CKDu .

The future

A solution for CKDu requires a joint scientific study involving multidisciplinary research teams to determine factors leading to the development of this disease. Experience from other countries indicates that this may take a long time. It is disheartening to observe that political decisions are made regarding scientific matters without consulting institutions that have been established by acts of parliament. Sri Lanka Association for the Advancement of Science or the Institute of Chemistry has not been consulted before making a decision on banning of glyphosate, which requires scientific advice. We hope that the glyphosate ban will be lifted and scientific discussions regarding the dangers will be held before making a decision.

———— * ————



Ethidium Bromide/ Acridine orange stained stem cells from umbilical cord blood.

Dananjaya Perera
(PhD student)
Dept. of Biochemistry & Molecular Biology, Faculty of Medicine, University of Colombo

Dates for your calendar…..

2017: August : CBSL Annual General Meeting and Scientific Symposium

2017: December 6-9: 26th FAOBMB conference; Kobe, Japan (abstract submissions 3-18 July).

Travel fellowships for young biochemists and molecular biologists not more than 40 years of age.
{Eligibility requires CBSL membership}
http://www.aeplan.co.jp/conbio2017/english/faobmb/index.html

Eligibility for CBSL membership

Full membership: if you are a confirmed senior lecturer or above in a Department of Biochemistry in a Sri Lankan university; hold a MPhil or PhD in a field of biochemistry /molecular biology; MSc in a field of biochemistry/molecular Biology with at least 5 yrs experience and 10 publications

Associate Membership: If you hold a BSc or MSc in a field of biochemistry/molecular biology

Student Memebership: If you are an undergraduate or postgraduate trainee in a field of biochemistry/ molecular biology

* Members of all categories are eligible for awards and fellowships of CBSL and FAOBMB

To

College of Biochemists Sri Lanka
Secretariate,
Dept. of Biochemistry
Faulty of medical Sciences
University of Sri Jayawardenapura,
Gangodawila, Nugegoda