Dwarka et al., Afr J Tradit Complement Altern Med. (2017) 14(2):253-262
doi:10.21010/ajtcam.v14i2.27

NEW INSIGHTS INTO THE PRESENCE OF BILIRUBIN IN A PLANT SPECIES *STRELITZIA NICOLAI* (STRELITZIACEAE).

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Abstract

Background: The fortuitous discovery of an animal pigment bilirubin found in the plant *Strelitzia nicolai* has opened an enormous number of questions regarding bilirubin's formation and its ultimate function in the human body.
Materials and Methods: A methodical review of bilirubin in humans and animals was carried out, information was gathered using published scientific journals, books and conference proceedings. Articles based on case studies of elevated levels of bilirubin were analysed thoroughly.
Results: Even though for numerous years bilirubin was assumed to be merely a desecrate product of the heme catabolic pathway by greatest, and a likely lethal compound at worst; statistics from the last few decades clearly shows that placidly high serum bilirubin levels are robustly related to have abundant beneficial effects on the human body.
Conclusion: This study reveals new insights into the presence of the only animal pigment found in *Strelitzia nicolai* arils, the potential advantages of bilirubin found in a plant and its therapeutic value indications. This review hopes to resuscitate researchers' credence regarding bilirubin as a toxic compound.

Key words: bilirubin, heme, biliverdin, biliverdin reductase, *Strelitzia nicolai*

Introduction

The catabolism of haem is the only natural progression in human beings that is colorimetric. This process would have been recognized by early man several thousand decades ago. After being injured directly on the skin a contusion that was black or purple (colours of haem) progressively changed to olive, the colour of biliverdin, and ultimately to yellow, the colour of bilirubin (Otterbein and Choi, 2000). Previously thought to be an "animal-only" pigment, in 2009, Florida International University researchers' (Pirone, 2010), made a breakthrough discovery in finding bilirubin in the arils of *Strelitzia nicolai* Regel and Koern, commonly known as the white bird of paradise. Bilirubin is a by-product from the secondary degradation of haem, the primary being biliverdin. In birds, reptiles and amphibians, biliverdin is the principal end product of heme dilapidation. In mammals, biliverdin endures an added metabolism to form bilirubin. This phenomenon has always mystified scientists.

Formation of Bilirubin in humans

Bilirubin is the terminal product of haem metabolism. Haem are established in haemoglobin, the major component of red blood cells. In humans, 250–400 mg of bilirubin are produced daily, of which more or less 20% is produced from non-haemoglobin sources (London et al., 1950). The conversion of haem to bilirubin is a two step procedure (Figure 1). First, microsomal heme oxygenase (HO), enzyme catalyses the oxidation of haem to a green tetrapyrrolic bile pigment, biliverdin. Subsequently biliverdin is transformed to bilirubin by biliverdin reductase (McDonagh, 2001). This reaction can occur in nearly every cell. An illustration is the formation of a bruise that undergoes different colours as it progressively mends i.e. red haem to green biliverdin to yellow bilirubin. Under standard physiological circumstances, the motion of haem oxygenase peaks in the spleen, where mature erythrocytes are sequestrated and annihilated.

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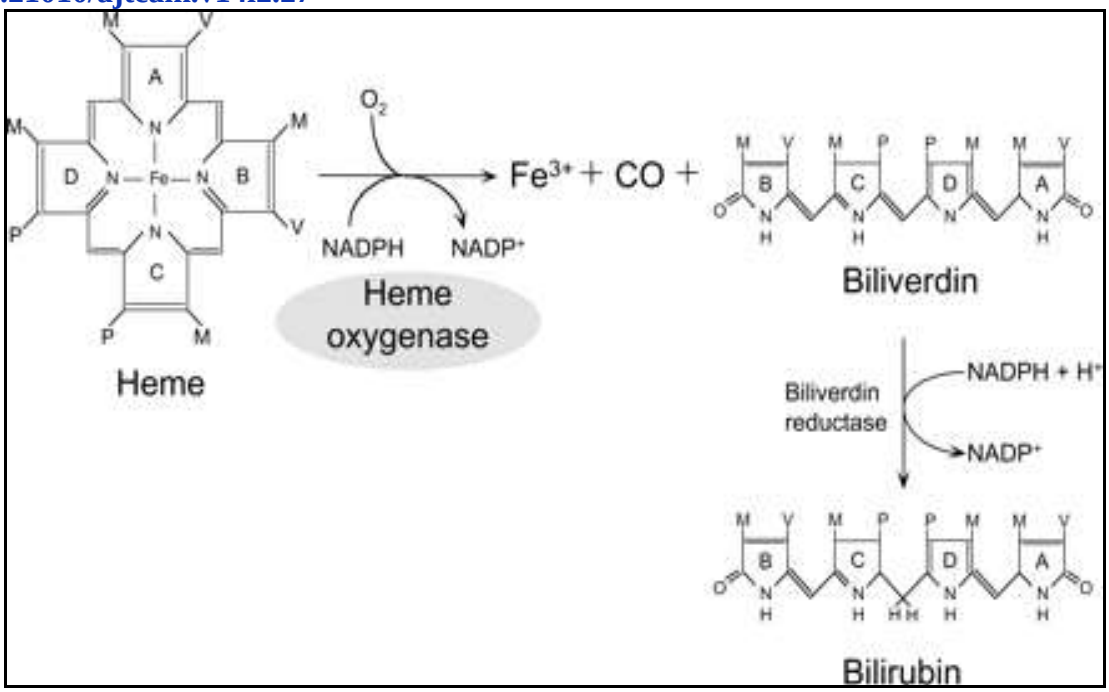

Figure 1: Enzyme-catalysed degradation of heme (Ramadori et al., 2000). Haem degradation starts by haem oxygenase-catalysed oxidation of the *a*-bridge carbon of haem that is converted to CO, directed to aperture of the tetrapyrrole ring which releases the iron molecule. The resulting biliverdin fragment is consequently abridged to bilirubin by cytosolic biliverdin reductase.

Metabolism of Bilirubin

Red blood cells are constantly undergoing a hemolysis course. The typical life-span of a red blood cell is 120 days. Whilst the red blood cells collapse, the haemoglobin is degraded or broken into globin, iron, and haem. The heme is first changed into biliverdin, which is quickly reduced to bilirubin. This process occurs in the reticuloendothelial cells of the liver, spleen, and bone marrow.

Haemoglobin, which is broken down to haeme in the spleen, is changed to form unconjugated bilirubin (Figure 2). This unconjugated bilirubin is insoluble in water, because of its intramolecular hydrogen bonding and attaches to albumin which is then sent to the liver. Inside the liver, bilirubin is conjugated through glucuronic acid by the enzyme glucuronyltransferase, rendering water soluble. Most of it goes into the small intestine together with the bile. About 95% of the bile created is taken back up by the liver. This bile is then resecreted by the liver into the small intestine. The procedure is identified as the enterohepatic circulation. About half of the conjugated bilirubin in the large intestine is exercised by gut bacteria to produce urobilinogen. This is then additionally oxidized to urobilin and stercobilin. Urobilin, stercobilin and their disintegrated products give faeces its brown colour (Kuntz, 2008).

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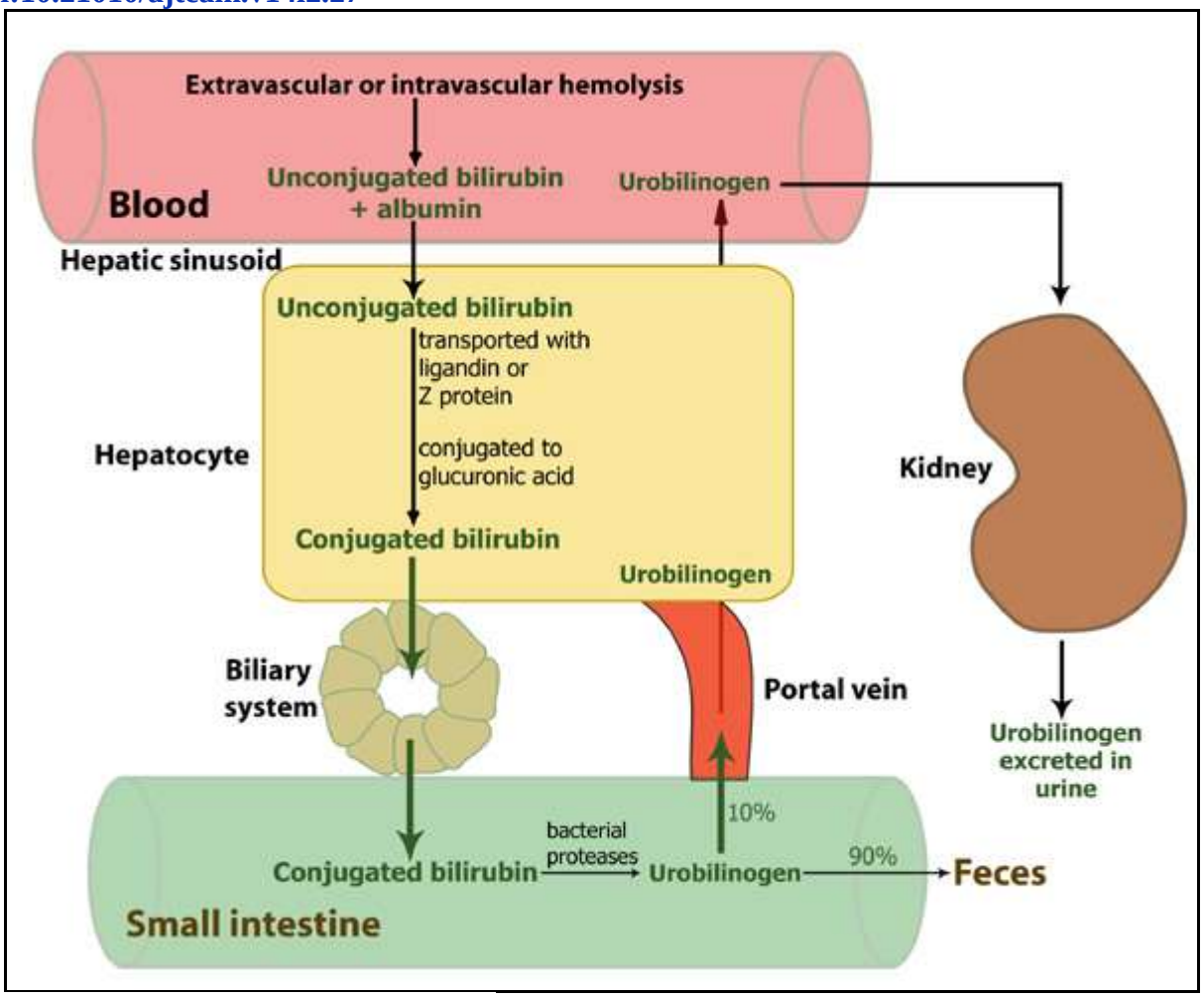

Figure 2: The enterohepatic circulation system (Sedlak and Snyder, 2004). Bilirubin is delivered to the liver, largely attached to albumin. In the liver, bilirubin is conjugated, excreted in the bile, modified in the gastrointestinal tract and largely lost via the faeces.

Bilirubin toxicity

Toxicity resulting from unconjugated hyperbilirubinemia has been known for more than a century, since the landmark study of Schmorl (1903) describing autopsy findings from 120 jaundiced infants (Hansen, 2000). Schmorl (1903) contributed immensely to the understanding of epidemiology and pathophysiology of neonatal jaundice and kernicterus.

Biliary efflux is the key route of removal of bilirubin and abate excretion will lead to toxicity of intracellular organelles and physiological processes. Amassing of bilirubin and bilirubin conjugates in human tissues generates jaundice, which is distinguished by soaring plasma bilirubin levels and deposition of yellow bilirubin tinctures in skin, sclerae, mucous membranes, and other less noticeable tissues (Lathe, 1972). Kernicterus is a form of brain damage caused by excessive jaundice.

The Gilbert syndrome is a harmless hereditary variation connected to unceasingly elevated plasma levels of unconjugated bilirubin (Ritter et al., 1992). This condition is emerges when the hepatic expression of the enzyme that conjugates bilirubin, glucuronosyltransferase type 1A1, is reduced. A more lethal inherited disorder affecting the metabolism of bilirubin is called the Crigler–Najjar syndrome. This uncommon disorder is a hereditary form of non-haemolytic jaundice, which is caused by mutations in the UGT1A gene encoding bilirubin glucuronosyltransferase. These produce elevated levels of unconjugated bilirubin which frequently directs to brain damage in infants (Ritter et al., 1992).

The Dubin–Johnson disease is an autosomal recessive condition which brings about an addition of conjugated bilirubin in the serum with no increase of liver enzymes (ALT, AST). The above disorder is fabricated by the insufficiency of canalicular multispecific organic anion transporter (cMOAT) gene (ABCC2/MRP2). This is connected to a flaw in the capability of hepatocytes to secrete conjugated bilirubin into the bile (Paulusma, et al., 1997). Mutations in the *SLCO1B1* and *SLCO1B3* genes cause the Rotor syndrome (non-icteric jaundice).

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The *SLCO1B1* and *SLCO1B3* genes code for creating proteins, called organic anion transporting polypeptide 1B1 (OATP1B1) and organic anion transporting polypeptide 1B3 (OATP1B3). The above proteins are established in the liver cells; these proteins convey bilirubin, starting from the blood heme to the liver. The *SLCO1B1* and *SLCO1B3* gene alterations that produces the Rotor syndrome lead to strangely small, nonfunctional OATP1B1 and OATP1B3 proteins or a nonexistence of these proteins. Lacking the occupation of these transfer protein, bilirubin is reducibly taken up by the liver and eliminated from the body (Strassburg, 2010).

Potential beneficial effects of bilirubin

For many years, unconjugated bilirubin (UCB) was considered a useless waste product of haeme catabolism, with no physiological function, but with potential toxicity. If this was a reality, then, why would the energy intense conversion of biliverdin to the water insoluble 'hazardous' bilirubin, which requires additional resources for shipping and secretion, occur? The breakthrough of finding the antioxidant and other properties of bilirubin has proposed that this conversion of biliverdin to bilirubin may actually be in reality, a progress in evolution (McDonagh, 2001). On the other hand Cornelius (1986), established that the enzyme biliverdin reductase existed even in Cyanobacteria, far prior to the existence of Eukaryotes on earth. Qin (2007), proposed that the inactivation of digestive proteases is the evolutionary dynamic strength for bilirubin preponderance. The quantity of digestive proteases secreted by the pancreas largely relies on the quantity of protein that is consumed. The quantity of digestive proteases secreted by the pancreas largely relies on the quantity of protein that is consumed. This presents a justification for the examination so as to bilirubin's principal genera be likely chosen carnivores or omnivores, whilst biliverdin-chief class tend to be herbivores (Howard and Yudkin, 1963).

Near the beginning of 1950, bilirubin was alleged to guard against the oxidation of lipids for instance, linoleic acid and vitamin A (Bernhard et al., 1954). Interestingly, evidence obtained in the last decade revealed a beneficial role of the molecule. Undeniably, physiological or modestly elevated serum levels of UCB have been shown to have a protective effect in several disorders, paradoxically even including neurodegenerative diseases. Protective effects of UCB rely on its antioxidant properties (Cuadrado and Rojo, 2008). Not only does bilirubin protect against oxidation, but it has also been reported to be beneficial against amylotrophic lateral sclerosis (Iłżecka and Stelmasiak, 2003), atopic dermatitis (Tsukahara et al., 1976), cancer (Temme et al., 2001) and Coronary artery disease (Hopkins et al., 1996). In diabetes mellitus, Han et al., (2010) discovered that an elevated intensity of serum bilirubin is related with a poorer hazard of the ailment.

Bilirubin as an antioxidant and cytoprotectant

Oxidative stress is damaging to life progression and is mainly accountable for maturation and age-related diseases. Oxidative stress is fundamentally a disproportion among the manufacturing of free radicals and the capability of the cells to counteract their damaging effects. For that reason, generally, the body is capable of producing a range of natural defence mechanisms in opposition to oxidative stress.

Thomas et al., (2008), found that the bilirubin pathway was a physiological cytoprotectant. Bilirubin principally defends against lipid peroxidation. This antioxidant effect impacts cell continued existence, As cell loss is additionally clearly amplified following reduction of bilirubin. Once bilirubin performs as an antioxidant, it is oxidized to biliverdin, biliverdin is then instantly reduced by biliverdin reductase to bilirubin.

Llesuy and Tomaro (1994), found that an increase in bilirubin production may perhaps be a reaction to early oxidative stress. They discovered that administering CO (II) to rats lead to oxidative stress, which precedes haem oxygenase stimulation. The introduction of this enzyme might be a method, during the amplification of bilirubin levels, to reduce the harm ignited by oxidative stress. During the infant period, most of the natural antioxidant level is fairly low compared to bilirubin. Bilirubin, which is deadly to neuronal cells at elevated levels, has been reported to have cytoprotective activity at lower concentrations (Breimer et al., 1995). Neonatal jaundice might also have a defensive outcome for the newborn moving for the first moment in time into an unhygienic environment. An investigation based on fit individuals assembled by low, in-between and elevated serum bilirubin intensity disclosed that high bilirubin levels guard against coronary flow reserve impairment, coronary microvascular dysfunction, and probably coronary atherosclerosis as well (Gullu et al., 2005). Biliverdin reductase is an evolutionarily preserved enzyme, renovating biliverdin to bilirubin, the potent physiological antioxidant. Bilirubin defends cells in opposition to high levels of hydrogen peroxide (H_2O_2), which oxidises bilirubin into biliverdin, which is then recycled flipside into bilirubin by biliverdin reductase (Baranano et al., 2002).

Bilirubin as an anti-cancer agent

Cancer is a term used for diseases in which atypical cells multiply without control and are able to invade other tissues. Cancer is a conglomerate of diseases. There are numerous types of cancers that are named according to the organ they affect.

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Zucker et al., (2004) researched a huge amount of people in the United States and with this they found that the probability proportion for colorectal cancer is condensed to 0.295 in men and 0.186 in women per 1 mg/dL increase in serum bilirubin intensity. Another study in Belgium by Temme et al., (2001) confirmed that there is a contrary connection among serum bilirubin concentration and cancer mortality.

The American Association for Cancer Research (2013) found that a 7.02 occurrence rate of lung cancer per 10,000 person-years for men with bilirubin concentrations of 0.68 mg/dL or a smaller amount compared with an prevalence rate of 3.73 amongst men whose bilirubin concentrations were 1.12 mg/dL or higher. This interprets into a 51 % amplification in the danger for developing lung cancer for patients with reduced bilirubin. Additionally, it was discovered that a lung cancer-specific mortality rate of 4.84 for men with the least amount of bilirubin compared with a mortality rate of 2.46 for men with the highest bilirubin concentration - a 59 % increase in lung cancer-specific death amongst persons with the lowest bilirubin concentration.

Bilirubin as a protectant in diabetes

Diabetes mellitus, the trademark of which consists of higher plasma glucose, is constantly coupled with amplified oxidative stress, as well as improved development of complex glycation end products. Overproduction of oxidizing particles causes the progressive loss of pancreatic β-cells, reducing insulin levels (Yamagishi et al., 2011).

Numerous studies have deduced that elevated bilirubin levels are contrariwise linked to the incidence of type 2 diabetes. Korean scientists reported considerable resistance of hyperbilirubinemic Gunn rats to initiated diabetes following intraperitoneal contact to Streptozotocin, compared to their normobilirubinemic littermates (Fu et al., 2010).

Cheriyath et al., (2010) confirmed that bilirubin might contribute a vital responsibility in glycemic management. Improved expression of haeme oxygenase-1, the enzyme responsible for the alteration of hemoglobin to bilirubin, is related with better insulin sensitivity and glucose metabolism. Additionally, serum bilirubin is contrariwise connected to insulin resistance since it amplifies the expression of glucose transporter-1 and the speed of glucose uptake.

Bilirubin as a protectant in cardiovascular disease

Cardiovascular diseases comprise hypertension, atherosclerosis, coronary artery illness and stroke. These are the leading causes of deaths in the world. Schwertner et al., (1994) established that a 50% reduction in total bilirubin was related to a 47% increment in the more rigorous coronary artery diseases.

Gilbert's syndrome is largely one of the frequent inherited genetic ailments. It is caused by an impaired glucuronyl transferase action leading from gentle to modest increase of serum bilirubin. Vitek et al., (2002) reported that the occurrence of ischemic heart condition in Gilbert's syndrome was established to be only 2%, in contrast with 12%, in control individuals.

Hopkins et al., (1996) established and completed a preceding study, that indicates, an increase in serum bilirubin level contained by the standard range is linked to a noteworthy and obvious decrease in coronary artery disease risk. Hopkins and Co-worker (1996) observed a 60% to 90% decrease in danger once serum bilirubin was in the higher two control quintiles compared with the lowest quintile for both men and women.

Bilirubin as a protector against neurological diseases

Takahashi et al., (2000) proposed that bilirubin is a powerful antioxidant in opposition to the cellular injure extracted by reactive oxygen species and contributes to the overall antioxidant network of the brain. Bilirubin has been established to be a powerful antioxidant in the brain, acting to forage peroxyl radicals as efficiently as α-tocopherol or vitamin E.

Iwasaki et al., (2005) found that a decreased concentration of serum bilirubin is correlated to an improved danger of neurodegenerative diseases. In addition, a low concentration of serum bilirubin is related to an increased risk of amyotrophic lateral sclerosis and psychological disorders, such as winter depression and schizophrenia.

The breakthrough of the only animal pigment (bilirubin) found in a plant

Strelitziaceae is a tropical monocotyledon family generating colourful bracteate inflorescences with woody capsular fruits that contain vibrantly colored arillate seeds. Pirone, (2010) discovered in the arils of *Strelitzia nicolai* (white bird of paradise) and *Strelitzia reginae* (the bird of paradise), the presence of bilirubin. The seeds contain an inner and outer integument, micropylar zone and an appendage in the form of dense tufts of a bright orange coloured aril (Van de Venter, 1986). Bilirubin was present in these arils as the primary pigment, and thus functions to produce vivid colours to attract animals.

Not like general plant pigments that decompose once the cell dies, aril pigments in the family endure for decades. Pirone, (2010) isolated the orange pigment from the arils of *Strelitzia nicolai*, and performed HPLC-ESMS,

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UV-visible, H NMR and C NMR investigation to conclude its chemical structure. The chemical properties of the compounds were atypical, and did not equal those of known colour classes (carotenoids, flavonoids, betalains, and the chlorophylls). The results revealed that the pigment was bilirubin-IX, an orange-yellow tetrapyrrole until that time known only in mammals and some other vertebrates.

The incidence of bilirubin discovery was not limited to *S. nicolai*. Two other species in the Strelitziaceae, *Phenakospermum guyanense* (A.Rich.) and *S. reginae*, contain aril pigments which co-eluted with genuine bilirubin in HPLC and had similar UV-visible spectra (Pirone, 2010). In the arils of the *Strelitzia* species, bilirubin was present as the principal pigment, and thus functioned to create colour. Previously, no tetrapyrroles were known to generate display colour in plants (Pirone, 2010). Bilirubin is therefore the initial creation of a further biosynthetic course, the tetrapyrrole pathway, to produce eye-catching colour in a plant reproductive constitution.

Tetrapyrroles are organic particles that had the utmost impact over the progression of existence for more than 4 billion years. These molecules play imperative roles in a variety of natural processes, including photosynthesis and respiration. Higher plants hold four classes of tetrapyrroles, namely, chlorophyll, haeme, siroheme, and phytochromobilin. Every one of the tetrapyrroles is derived from an ordinary biosynthetic pathway (McDonagh, 2001). The chief location of tetrapyrrole biosynthesis in plants transpires in plastids. Tetrapyrrole biosynthesis is dominantly regulated by acclimation to environmental conditions.

Pathway of tetrapyrrole biosynthesis

The initial phase in the tetrapyrrole biosynthesis is the production of 5-aminolaevulinic acid ALA via two probable routes: (1) condensation of succinyl CoA and glycine (C4 pathway) using ALA synthase or (2) decarboxylation of glutamate (C5 pathway) via three diverse enzymes, glutamyl-tRNA synthetase to charge a tRNA with glutamate, glutamyl-tRNA reductase to decrease glutamyl-tRNA to glutamate-1-semialdehyde (GSA), and GSA aminotransferase to catalyse a transamination response to manufacture ALA (Grimm, 2003).

The next period is to change ALA to uroporphyrinogen III. This is attained by the achievement of three enzymes in a single general pathway: porphobilinogen (PBG) synthase or ALA dehydratase, to condense two ALA fragments to produce porphobilinogen; hydroxymethylbilane synthetase, to polymerise four PBG molecules into preuroporphyrinogen (tetrapyrrole structure); and uroporphyrinogen III synthetase to connect two pyrrole components as one to yield uroporphyrinogen III (Grimm, 2003). To manufacture haem and chlorophyll, uroporphyrinogen III requires to be decarboxylated into coproporphyrinogen III by the act of uroporphyrinogen III decarboxylase.

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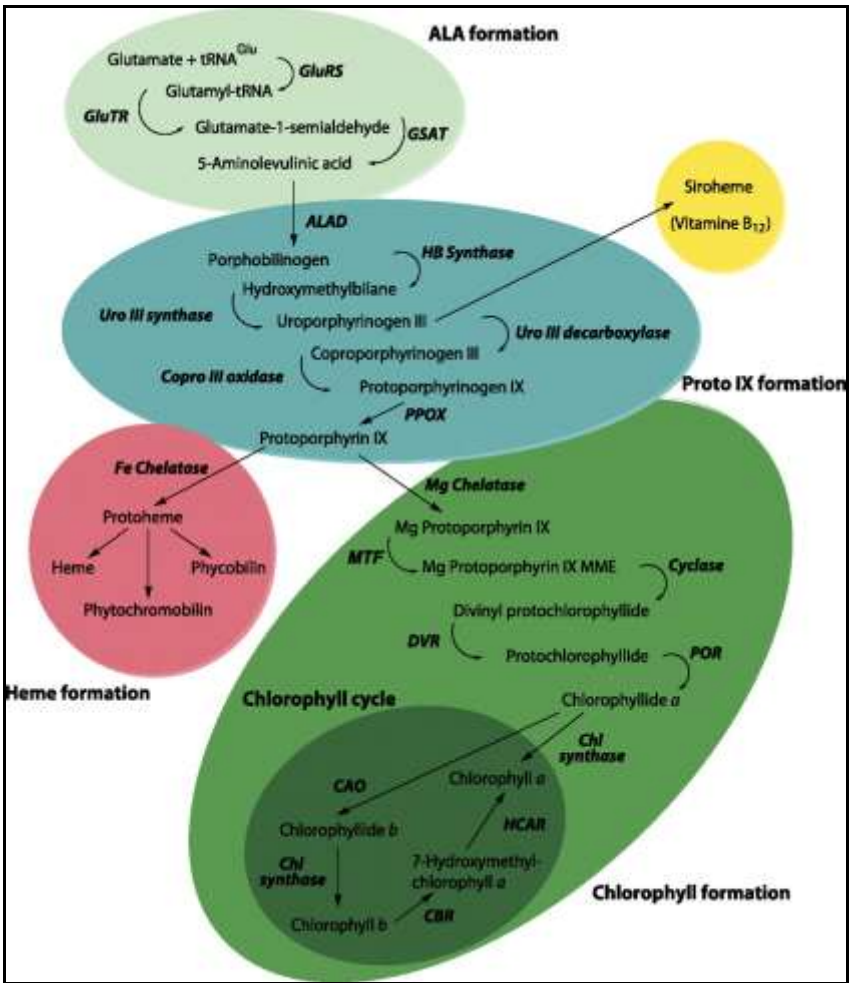

Figure 3: Biochemical pathway of plant tetrapyrrole biosynthesis. The global precursor of all tetrapyrroles, 5-aminolevulinic acid (ALA), is produced from glutamate. ALA is additionally processed to protoporphyrin IX before the approach branches into haem and chlorophyll biosynthesis (Czarnecki and Grimm, 2012).

Differences and similarities of the tetrapyrrole metabolic synthesis in animals and plants

The enzymatic steps are the most important differences in the pathways. Another major difference is the two routes of ALA synthesis, which developed independently during evolution (Tanaka and Tanaka, 2007). These two paths differ in the commencing metabolites. The earliest path is found in all bacteria and plants. It starts with glutamate and three enzymes needed to form ALA. In animals and yeasts, a succinyl-CoA-glycin entry was elucidated with one enzyme to form ALA (Mochizuki, 2010).

The configuration of haem in all organisms is attained by the chronological act of eight discernible enzymes. The plant tetrapyrrolic pathway additionally includes several bicurations for synthesis of different products (Tanaka and Tanaka, 2011).

A major distinction of plant and animal tetrapyrrolic biosynthesis pathway is the different subcellular localization of the pathway. The animal metabolic chain is spatially separated within the cell (Grimm, 2003). Four enzymes are located in the cytoplasm and another four are present in the mitochondria. In plant, the whole metabolic pathway for chlorophyll is located in the chloroplasts. Here the synthesis takes place in plastids and in the mitochondria (Jensen et al, 1999).

Can bilirubin's newfound status be harnessed therapeutically?

Despite a century of research, several clinical areas of bilirubin remain poorly understood, controversial, or unrecognized. The breakthrough in finding profuse amounts in the orange mop like turfed arils of the seeds of the *Strelitzia spp* and *Phenakospermum guyanense* has opened up new opportunity for the possible insight into the evolutionary reason for the baffling biosynthesis in humans.

It has been acknowledged that tropical rainforest environments are so nutrient poor that humans could not survive within, without access to food crops. Recent investigations into the diets of Amazonian forest dwellers have discovered

doi:10.21010/ajtcam.v14i2.27

that their diets consist primarily of palm nut and the seeds are ground, powdered and made into a porridge. Arils are also chewed and the juices are consumed. Ambiguously, these forest dwellers live very extended and healthy lives (Milton et al, 1991). Could it be due to the bilirubin found in the arils that forms part of their primary diet? If so, this could be the cytoprotective effect suspected to be the underlying characteristics of bilirubin. Sunbirds are the main pollinators of the white bird of paradise tree. Birds don't produce bilirubin, they produce biliverdin. Therefore, this could be the reason they consume the bilirubin found in the arils. In fact, an elevated level of bilirubin, in birds after consumption does not impair them in anyway. Therefore, there is rationale to believe that bilirubin found in these arils could have a possible protective effect.

The human body is a multifaceted being which has the aptitude to mend itself if listened to and concerned for correctly. However in the modern world, the variables are almost infinite. It is a fact that people of the world today are having more problems with their health than ever before. It is for this reason that scientists nowadays are looking at our pasts in order to produce medicines for the future since for hundreds of years man has used plant extracts to cure ailments. This study makes us believe that there is now strong experimental and theoretical support for the claim that bilirubin has a host of beneficial effects.

The most important piece of evidence on the subject of bilirubin as a possible beneficial product, lies in the fact, that in most animal cells the degradation of haemoglobin, if stopped an action prior, with a green, soluble fragment called biliverdin, the desecrate would be simply emitted with no danger of detrimental accumulation. However, as an alternative to stopping at biliverdin, for the most part, animal cells persist on to formulate bilirubin by means of biliverdin reductase. If the only result of bilirubin in high amounts is toxicity, then it's illogical that animals would produce it using a high energy consuming step.

Conclusion

In this review, the investigational as well as clinical studies on the relationships between bilirubin and its protective effect on certain aspects of the human body were conferred. These studies collectively illustrates that low serum bilirubin levels are linked with an increased risk of several pathological conditions; whereas, mildly elevated serum bilirubin levels provide protection. It is for this reason we can therefore hypothesize that if the bilirubin found in the human body has a protective effect against numerous conditions, perhaps the bilirubin found in the arils of *S. nicolai* will have similar effects and could be harnessed therapeutically to modulate certain pathological conditions. Clearly, detailed studies to investigate this potential further emerges necessary, and will unquestionably provide much needed substantiation.

References

1. American Association for Cancer Research (AACR) (2013). Novel serum biomarker bilirubin predicted lung cancer risk in smokers.
2. Baranano, D.E., Rao, M. and Ferris, C.D. (2002). Snyder SH. Biliverdin reductase: a major physiologic cytoprotectant. *Proc. Natl. Acad. Sci. U.S.A*. 99: 16093–16098.
3. Bernhard, K., Ritzel, G. and Steiner, K.U. (1954). On a biological significance of bile pigments: bilirubin and biliverdin as antioxidants for vitamin A and essential fatty acids. *Helv Chim Acta*. 37: 306–31.
4. Breimer, L.H., Wannamethee, G. and Ebrahim, S. (1995). Serum bilirubin and risk of ischemic heart disease in middle-aged British men. *Clin Chem*. 41: 1504–1508.
5. Cheriyath, P., Gorrepati, V.S., Peters, I., Nookala, V., Murphy, M.E, Srouji, N. and Fischman, D. (2010). High total bilirubin as a protective factor for diabetes mellitus: An analysis of NHANES data from 1999-2006. *J Clin Med Res*. 2: 201–206.
6. Cornelius, C.E. (1986). Comparative bile pigment metabolism in vertebrates. In: Ostrow JD, ed. 601–647.
7. Cuadrado, A. and Rojo, A.I. (2008). Heme oxygenase-1 as a therapeutic target in neurodegenerative diseases and brain infections. *Curr Pharm Des*. 14: 429-442.
8. Czarnecki, O. and Grimm, B. (2012). Post-translational control of tetrapyrrole biosynthesis in plants, algae, and cyanobacteria. *J. Exp. Bot*. 63 (4): 1675-1687.
9. Fu, Y.Y., Kang, K.J., Ahn, J.M., Kim, H.R., Na, K.Y., Chae, D.W., Kim, S. and Chin, H.J. (2010). Hyperbilirubinemia reduces the streptozotocin-induced pancreatic damage through attenuating the oxidative stress in the Gunn rat. *Exp. Med*. 222: 265–273.
10. Grimm B. (2003). Regulatory mechanisms of eukaryotic tetrapyrrole biosynthesis. The porphyrin handbook: iron and cobalt pigment, biosynthesis, structure and degradation. 2003. Volume 12: 1-2.

11. Gullu, H., Erdogan, D., Tok, D., Topcu, S., Caliskan, M., Ulus, T. (2005). High serum bilirubin concentrations preserve coronary flow reserve and coronary microvascular functions. *Arteriosclerosis, Thrombosis and Vascular Biology*. 25: 2289–2294.
12. Han, S.S., Na, K.Y., Chae, D.W., Kim, Y.S., Kim, S. and Chin, H.J. (2010). High serum bilirubin is associated with the reduced risk of diabetes mellitus and diabetic nephropathy. Tohoku J. *Exp. Med.* 221: 133–140.
13. Hansen, T.W.R. (2000). Pioneers in the scientific study of neonatal jaundice and kernicterus. *Pediatrics*. 106: 2-15.
14. Hopkins, P.N., Wu, L.L., Hunt, S.C., James, B.C., Vincent, M.G. and Williams, R.R. (1996). Higher Serum Bilirubin Is Associated With Decreased Risk for Early Familial Coronary Artery Disease. *Arterioscler Thromb Vasc Biol*.16(2): 250-5.
15. Howard, F. and Yudkin, J. (1963). Effects of dietary change upon the amylase and trypsin activities of the rat pancreas. *Brit. J. Nutr*. 17: 28-37.
16. Iłżecka, J. and Stelmasiak, Z. (2003). Serum bilirubin concentration in patients with amyotrophic lateral sclerosis. *Elsevier*. 8: 20-954.
17. Iwasaki, Y., Igarashi, O., Iwasa, Y., Hirano, K., Satoh, R., Iwamoto, K., Kawase, Y., Aoyagi, J., Ichikawa, Y., Kawabe, K. (2005). Bilirubin and amyotrophic lateral sclerosis. *Clin. Neurol. Neurosurg*. 5: 107- 160.
18. Jensen, P.E., Gibson, L.C. and Hunter, C.N. (1999). ATPase activity associated with the magnesium-protoporphyrin IX chelatase enzyme of Synechocystis PCC6803: evidence for ATP hydrolysis during Mg2+ insertion, and the MgATP-dependent interaction of the ChlI and ChlD subunits. *Biochem. J*. 339: 127–134.
19. Kuntz, E. (2008). Hepatology: Textbook and Atlas. Germany: Springer. 38, ISBN 978-3-540-76838-8.
20. Lathe, G.H. (1972). The degradation of haem by mammals and its excretion as conjugated bilirubin. *Essays Biochem*. 8: 107-148.
21. Llesuy, S.F. and Tomaro, M.L (1994). Heme oxygenase and oxidative stress. Evidence of involvement of bilirubin as physiological protector against oxidative damage. *Biochimica et Biophysica Acta*. 1223: 9-14.
22. London, I.M., West, R. and Shemn, D. (1950). On the origin of bile pigment in man. *J Biol Chem*. 184: 351–358.
23. McDonagh, A.F. (2001). Turning green to gold. *Nat Struct Biol*. 200: 98–200.
24. Milton, K., Knight, C.D. and Crowe, I. (1991). Comparative aspects of diet of Amazonian forest dwellers. Phil. Trans. 334: 253-263.
25. Mochizuki, N., Tanaka, R., Grimm, B., Masuda, T., Moulin, M. and Smith, A.G. (2010). The cell biology of tetrapyrroles: a life and death struggle. *Trends Plant Sci*. 15: 488–498.
26. Otterbein, L.E and Choi, A.M. (2000). Heme oxygenase: colors of defense against cellular stress. *J Physiol Lung Cell Mol Physiol.* 279(6): 29-37.
27. Paulusma, C.C., Kool, M. and Bosma, P.J. (1997). A mutation in the human canalicular multispecific organic anion transporter gene causes the Dubin–Johnson syndrome. *Hepatology.* 25: 1539–1540.
28. Pirone C. (2010). "Bilirubin: an Animal Pigment in the Zingiberales and Diverse Angiosperm Orders" (2010). FIU Electronic Theses and Dissertations. Paper 336. http://digitalcommons.fiu.edu/etd/336
29. Qin, X. (2007). Inactivation of digestive proteases by deconjugated Bilirubin: the possible evolutionary driving force for bilirubin or biliverdin predominance. *Gut.* 56(11): 1641–1642.
30. Ramadori, G., Immenschuh, S. and Medizin, I.Z. (2000). Gene regulation of heam oxygenase-1 as a therapeutic target. *Abteilung Gastroenterologie und Endokrinologie*. 48: 53—57.
31. Ritter, J.K., Yeatman, M. T., Ferreira, P. and Owens, I.S. (1992). Identification of a genetic alteration in the code for bilirubin UDP- Glucuronosyltransferase in the UGT1 gene complex of a Criler-Najjar type 1 patient. *J Clin Invest*. 90: 150-155.
32. Schwertner, H.A., Jackson, W.G. and Tolan, G. (1994). Association of low serum concentration of bilirubin with increased risk of coronary artery disease. *Clin. Chem.* 40: 18–23.
33. Schmorl G. (1903). Zur Kenntnis des ikterus neonaturum, insbesondere der dabei auftretenden gehirnveranderungen. *Verh Dtsch Ges Pathol*. 6 :109–15.
34. Sedlak, T.W., Snyder, S.H. (2004). Bilirrubin benefits: cellular protection by a biliverdin reductase antioxisant cycle. *Pediatrics* 113: 1776-82.
35. Strassburg, C.P. (2010) Hyperbilirubinemia syndromes (Gilbert-Meulengracht, Crigler-Najjar, Dubin-Johnson, and Rotor syndrome). *Clin Gastroenterol*. 5, 555-571.
36. Takahashi, M., Dore, S., Ferris, C.D., Tomita, T., Sawa, A., Wolosker, H., Borchelt, D.R., Iwatsubo, T., Kim, S.H., Thinakaran, G., Sisodia, S.S. and Snyder, S.H. (2000). Amyloid precursor proteins inhibit heam oxygenase activity and augment neurotoxicity in Alzheimer's disease. *Neuron.* 28: 461–473.
37. Tanaka, R. and Tanaka, A. (2011). Chlorophyll cycle regulates the construction and destruction of the light-harvesting complexes. *Biochim. Biophys. Acta*. 180 : 968–976
38. Tanaka, R., Tanaka, A. (2007). Tetrapyrrole biosynthesis in higher plants. *Annual Review of Plant Biology*. 58: 321–346.
39. Temme, H.M.E., Zhang, J., Schouten, G.E. and Kesteloot, H. (2001). Serum bilirubin and 10-year mortality risk in a Belgian population. *Cancer Causes and Control*. 12: 887-894.

doi:10.21010/ajtcam.v14i2.27
40. Thomas, W., Sedlaka, M.S., Higginsonb, D.S., Paulb, B., Julurib, K.R. and Snydera, S.H. (2008). Bilirubin and glutathione have complementary antioxidant and cytoprotective roles. *Pubmed*. PMCID: PMC266404.
41. Tsukahara, H., Shibata, R., Ohshima, Y., Sato, S., Ohta, N., Hiraoka, A., Nishima, S. and Van de Venter, H.A. (1976). A note on ovular outgrowths in the species of strelitzia. *Journal South African Botany*. 42: 37-39.
42. Van de Venter, H.A. (1986). Some characteristics of leaf and inflorescence production in Strelitzia reginae. *Journal South African Botany*. 4: 305-31.
43. Vitek, L., Jirsa, M., Brodanova, M., Kalab, M., Marecek, Z., Danzig, V., Novotny, L., Kotal, P. (2002). Gilbert syndrome and ischemic heart disease: a protective effect of elevated bilirubin levels . *Atherosclerosis*. 160: 449–456.
44. Yamagishi, S.I., Maeda, S., Matsui, T. and Ueda, S. (2011). Fukami K and Okuda S. Role of advanced glycation end products (AGEs) and oxidative stress in vascular complications in diabetes. *Biochim. Biophys. Acta*. 20-27.
45. Zucker, S.D., Horn, P.S. and Serman, K.E. (2004). Serum bilirubin levels in the U.S. population: gender effect and inverse correlation with colorectal cancer. *Hepatology*. 40: 827–835.