A REVIEW ON PHARMACOLOGICAL ACTIVITIES OF BETaine
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Abstract

Betaine is an important phytoconstituent found in plant Beta vulgaris. It is found abundantly in various common foods like beets, spinach and cereals, whereas wheat is its major source in human diet. Chemically, betaine is termed as trimethylglycine. It plays an important physiological role in the body. It has found to be an osmolyte and it participates in methionine–homocysteine cycle as a methyl group donor due to which it possesses many physiological activities. It has widely been studied for its promising antioxidant properties, regulating energy metabolism to relieve chronic inflammation, mitigation of endoplasmic reticulum stress and apoptosis, canonical and non-canonical inflammasome-mediated processing of IL-1β, against neurodegenerative diseases, anticonvulsant effects, reducing the total homocysteine content in brain and its role in performance and action. The present article firstly aims to review the pharmacological effects including neuroprotective effects, physiological effects of betaine including effects on performance and human body composition, secondly to study the safety concerns of betaine and its future prospects.

Keywords: Betaine, osmoprotectant, methyl group donor, antioxidant, anti-inflammatory and neuroprotection.

1. Introduction

Betaine is an important phytoconstituent found in plant Beta vulgaris. It was first identified during the nineteenth century in Beta vulgaris. After that, it was also found in many other organisms like beets, spinach, wheat germ, wheat bran, some aquatic species and microorganisms (Craig, 2004). The amount of intake of betaine in diet relies on various sources of betaine and methods of cooking (Zeisel et al., 2003).

Dietary intake of betaine plays an important part in its content present in the body. Betaine intake of 9–15 g daily has found to be safe for health. It gets distributed primarily to the brain, liver and kidneys (Craig, 2014). Betaine is also synthesized in the body from choline. Various studies have reported that its high concentrations in neonates indicate that the mechanism of synthesis of betaine in the body is highly effective (Zeisel et al., 2013; Davies et al., 1988).

Chemically, betaine is termed as trimethylglycine (trimethyl derivative of the amino acid glycine). At neutral pH, it has found to exist as the (CH₃)₃N⁺CH₂COO⁻ zwitterion and is found to be highly soluble in water (Pummer et al., 2000). Betaine has found to be present as modified amino acid in many foods and is found in high content particularly in whole grains (Craig et al., 2004). The intake of betaine and its plasma levels have found to be inversely correlated with the markers of various metabolic syndromes (Detopoulou et al., 2008; Konstantinova et al., 2008). It is found abundantly in various common foods like beets, spinach and cereals, whereas wheat is its major source in human diet (Hedemann et al., 2015; Corol et al., 2012).

Betaine participates in methionine–homocysteine cycle as a methyl group donor due to which it possesses many physiological activities (Zhang et al., 2016; Jin et al., 2015). Betaine has found to be an organosoluble and compatible solute which contributes to osmotic adjustment in animals, plants, bacteria, algae and fungi (Hedemann et al., 2015; Corol et al., 2012).

In recent years, betaine has widely been studied for its antioxidant properties. It has been reported to relieve the oxidative pressure and thereby increasing the productivity of various crops, such as wheat, barley, sorghum, beans and soybean in stressful conditions (Bharwana et al., 2014; Osman, 2015). So, betaine is found to be considered as a promising antioxidant for alleviating the stress like chronic heat and enhanced the quality of meat in poultry (Akhaven-Salamat and Ghasemi, 2016; Alirezaei et al., 2012).

Additionally, betaine has found to exhibit a protective effect against the inducers of oxidative stress such as levodopa and ethanol in organs like brain, liver, brain, stomach, ovaries and kidneys of rats (Alirezaei et al., 2014; Alirezaei et al., 2015; Alirezaei, 2014; Ahn et al., 2014; Alirezaei et al., 2012).

Betaine plays an important physiological role in the body. Principally acting as an organic osmolyte it protects the cells from osmotic stress. The synthesis of betaine has been found to be triggered in cells when they are exposed to high temperature or salinity, temperature stress or drought and it gets accumulated in the cells. As a compatible osmolyte, it has found to increase the retention of water in cells. It has a protective effect for intracellular enzymes against osmotic inactivation (Alirezaei et al., 2015).

Betaine has been reported to protect the vital organs involving heart, kidney and brain. Betaine has been reported as a neuroprotective agent against experimentally induced oxidative damage and Benserazide mediated hyperhomocysteinemia in brain tissue of rats (Alirezaei, 2004). It has also been found to protect the rats from ethanol-induced oxidative stress and to reduce the total homocysteine (Hcy) content in the rat brain (Alirezaei et al., 2015). So, it is suggested that betaine may be therapeutically effective as a neuroprotective agent in neurodegenerative diseases including Parkinson disease (Alirezaei et al., 2015, Lawson and Levy, 2006).
Therefore, physiologically in humans, betaine has found to serve a dual role firstly, in the transmethylation of Hcy as a methyl donor and secondly, in maintaining fluid balance as an osmolyte. Betaine has also been proposed for its ergogenic potential firstly by Borsook et al. (1952). The general strength and endurance of poliomyelitis patients was found to be improved when they were given supplements of betaine–guanidinoacetate Borsook et al. (1952). After that it was reported to improve hydration and lactate metabolism in horses fatigued with exercise (Warren et al., 1999). Later, several human studies investigating betaine for ergogenic effects on power and strength have been carried out that reported betaine to improvement anaerobic metabolism in humans (Armstrong et al., 2005).

Betaine has been used in pigs and chickens for improving the yield of lean meat, this pushed some researchers to carry out studies investigating betaine for its potential to improve human body composition (Eklund et al., 2001). It has been reported in a pharmacokinetic study that in healthy volunteers and homocystinuria patients that the plasma concentrations of betaine were found to sharply increase after oral administration betaine. Betaine can be filtered freely by the kidneys and then reabsorbed back into the circulation, so excretion is primarily in sweat rather than in urine (Lever et al., 2004; Craig et al., 2010). Accumulation of betaine in body depends on its specific transporters, and it distributes primarily to the brain, liver, and kidneys (Craig, 2004). It distributed rapidly and its apparent volume of distribution is relatively large. A biexponential decline was observed in the plasma concentration–time curve in a two-compartment model. Betaine elimination from the body was found to be rather slow. Throughout the pharmacokinetic study, all the routine laboratory data was found to remain unchanged (Schwahn et al., 2003).

3. Pharmacological Actions of Betaine

Although in most of the tissues such as those of the kidneys and brain betaine has been reported to be utilized as an osmoprotectant, primarily it has found to playing an important role in liver metabolism as a methyl group donor (Knight et al., 2017; Day et al., 2016).

3.1 Betaine as an Osmoprotectant

Osmoprotectants, in contrast to inorganic salts are small organic compounds that are highly soluble and accumulate largely in cells without interfering with any of the cell functions; osmoprotectants protect the cells from osmotic stress and damage (Yancey, 2005). Hyperosmosis is the condition that can cause efflux of water and a concomitant reduction in the volume of cell, which is detrimental to the survival of cell (Neuhofer and Beck, 2005). Thus, it is essential for different osmoprotectants, such as betaine, taurine, and sorbitol, to accumulate in cells in an attempt of balancing hyperosmosis and protecting the cells from shrinkage and thereby death (Kempson et al., 2014; Dasgupta and Kishore, 2017; Mcneil et al., 1999; Veis et al., 1991; Bucolo et al., 2017). In contrast to inorganic salts such as Na+ and urea and other osmolytes, betaine has been found to reduce the salvation of proteins by water molecules thus making the native protein structures stabilize (Adamczak et al., 2016). Betaine has also been found to increase the volume of cytoplasm and content of free water of cells to prevent cell shrinkage in conditions like hyperosmosis and to inhibit the apoptosis of various native proteins that can be induced by hyperosmosis (Ratriyanto et al., 2009; Horio et al., 2001). Having these advantages, betaine can additionally be used to counter the pressure in hypertonic tissues e.g. the levels of the betaine–GABA (γ-aminobutyric acid) transport system i.e. GAT4/BGT1 in the basolateral plasma membrane, in kidneys, in hypertonic conditions for obtaining more betaine whereas the levels of BGT1 have found to be low in normal physiological conditions. This transporter is primarily present in MDCK (Madin–Darby canine kidney) cells cytoplasm (Kempson et al., 2014).

3.2 Betaine as a Methyl group Donor

Betaine, as a methyl group donor participates in methylation, such as that of protein and DNA which is an essential biochemical process in found in animals. This has been shown by the previous studies that the methyl group donors availability influences the levels of methylation (Zeisel, 2017). Betaine, choline, and methionine have been acknowledged to be the most important methyl group donors found in diets. Nevertheless, methionine is primarily a substrate for the synthesis of proteins, and choline is primarily contributing in the formation of neurotransmitters and the cell membrane. Betaine plays a major role in the transmethylation reaction that principally occurs in kidney cells and mitochondria of liver. In this transmethylation reaction, a methyl group donated by betaine is added to homocysteine in formation of methionine. Methionine is then subsequently converted to DMG (dimethylglycine) (Williams and Schalinske, 2007). DMG is then degraded to sarcosine and ultimately to glycine. Another important enzyme is methionine synthase catalyzing the formation of methionine from homocysteine in that methyl group is donated by N5-methyltetrahydrofolate. These are very important reactions occurring in animals as they are conserving methionine and detoxifying the homocysteine, that is is involved in various cardiovascular disease (Ueland and Refsum, 1989) S-adenosylmethionine (SAM) is principal methylating agent formed from methionine catalyzed by methionine adenosyltransferase (Barak et al., 1996). SAM is then transformed into S-adenosylhomocysteine (SAH) after demethylation. The SAM and SAH thus formed then affect the various SAM-dependent methyltransferases that are found to be concerned with the repair progress of protein, metabolism of lipid GTPase activity and various inter protein interactions (Yamamoto et al., 1998; Nishimakimogami et al., 2002; Boisvert et al., 2003; Rando, 1996; Kramer et al., 2003; Kim et al., 2015). One SAH molecule is subsequently hydrolyzed to one homocysteine molecule and one adenosine molecule by the action of SAH hydrolase. The methionine cycle is constituted by all of these reactions. Another vitamin B-6-dependent enzyme, cystathionine β-synthase has been found to catalyze the transformation of cystathionine from...
homocysteine via a pathway termed as transsulfuration. The catabolism of homocysteine in this pathway led to the increased production of glutathione, taurine, and the other metabolites (Mosharov et al., 2000; Jung et al., 2003; Meng et al., 2013). Betaine as a dietary supplement has been reported to impact various sulfur amino acids (Craig, 2004). The availability methionine and SAM is increased effectively by such dietary supplements (Cholewa et al., 2014; Lawsonyuen and Levy, 2006).

3.3 Anti-Inflammatory Effects of Betaine

Inflammation is protective immune response that is primary and essential process of healing of wound and host defence. However, inflammation may be serve as pathogenesis of other diseases if it is prolonged and excessive. Natural products could be a good strategy for such treatments and they reduce the intensity of the inflammatory reaction (Jin et al., 2013). In recent years, response of betaine to inflammation has become a sparked heated debate. The review is discussing the various mechanisms involved in the anti-inflammatory effects of betaine.

3.4 Betaine against Oxidative Stress

Oxidative metabolism occurs primarily in mitochondria, and the reactive oxygen species (ROS) that are the by-products of reactions involved in oxidative metabolism are formed in mitochondria; in particular. Two detoxification systems have been found in body under normal physiological conditions, for the clearance of ROS and free radicals. These are antioxidant agents and antioxidant enzymes (Sies, 1997; Davies, 1995). The examples of detoxification agents include superoxide dismutase (SOD), Catalase, GSH and melatonin (Tsai and Huang, 2015; Tan et al., 2007; Dumaswala et al., 2001). Excessive production of ROS is dangerous to cells as the stability of nucleic acids, associated proteins and lipid membrane is affected by them. So, high ROS levels bring about pathological changes and inflammation (Freitas et al., 2016).

Sulfur amino acids (SAA) including methionine, homocysteine, cysteine, SAH and SAM have been reported to be involved in many important metabolic pathways including transmethylation reactions, protein synthesis and GSH synthesis (Zhang et al., 2017). Hyperhomocysteinemia have been reported by various studies to be ultimately involved in induction of oxidative stress and apoptosis (Almashhadany et al., 2015; Baggott and Tamura, 2015). Studies have reported that betaine stimulates the formation of methionine from homocysteine and therefore it directly influences the concentrations of homocysteine to regulate the concentrations of SAA (Barak et al., 1997). Betaine, as an alternate methyl donor has been used in ethanol-fed Wistar rats and it improved the activity of BHMT in the generation of methionine and SAM and found to remove homocysteine in their livers (Barak et al., 1997; Ji and Kaplowitz, 2003). However, a decrease or no change in BHMT expression was observed in C57B6 mice rather than increase (Shinohara and Ji, 2010). As betaine has been involved in conversion of homocysteine to methionine so it may said that the concentrations of methionine are in close relation with those of betaine. Methionine is importantly involved in antioxidation as methionine relieves oxidative stress via chelation, and it can be used for synthesis of GSH by hepatocytes (RC et al., 2001; Martínez et al., 2017).

3.5 Betaine inhibits the NF-κB Signaling Pathway

Many genes that are involved in the process of inflammation are controlled by the NF-κB (transcription factor nuclear factor-κB) signalling pathway. The genes involved in inflammation include the TNF-α (tumor necrosis factor- alpha), pro-inflammatory cytokines, IL-23 (interleukin 23) and IL-1β (interleukin 1 beta). NF-κB is activated chronically in many diseases of inflammatory origin (Monaco et al., 2004; Aravilli et al., 2017; Schuliga, 2015). Therefore, NF-κB signalling pathway has known to be a very important candidate for treatment of inflammation. Betaine has been reported by many researchers to suppress the activity of NF-κB and various genes involved in inflammation (Go et al., 2005; Lee et al., 2013; Yi and Kim, 2012) e.g. Betaine treatment in aged kidneys has been found to suppress the activity of NF-κB and various related genes expression including VCAM-1 (vascular cell adhesion molecule-1), TNF-α and ICAM-1 (intracellular cell adhesion molecule-1), iNOS (inducible nicotinic oxide synthase), COX-2 (cyclooxygenase-2) and iNOS (inducible nicotinic oxide synthase) (Go et al., 2005). It may be noted that in this and another studies related with atherogenesis, it has been found the authors that betaine inhibited the NF-κB signalling pathway by the suppression of two important kinases firstly, MAPKs (mitogen-activated protein kinases) and secondly, NIK/IKK (nuclear factor-induced kinase/IKB kinase) (Go et al., 2005; Lee et al., 2013). NIK/IKK have been known to relieve the inhibition of IκB and initiating the NF-κB transcriptional activation (Je et al., 2004). MAPKs have been studied for their involvement in inflammatory process and are responsive to the expression of pro-inflammatory cytokine (Kyriakis and Avruch, 2012). Betaine has been found to exert mechanistical effects by inhibiting NF-κB and ROS, and by maintaining the levels of thiol particularly GSH (Go et al., 2007). Some of the important upstream signalling molecules such as TLRs (Toll-like receptors) and LPS (lipopolysaccharide, specifically TLR-4 activator) culminating to activate NF-κB have also been found to be inhibited by betaine. In vitro study performed in RAW 264.7 murine macrophage cells reported the prevention of LPS-induced activation of NF-κB with betaine treatment (Kim et al., 2014). Betaine treatment in another study has shown the improvement of neural injury in hypothalamus through the inhibition of pathway, TLR-4/NF-κB. This is also suggested by this study that betaine was able to inhibit the expression of histone deacetylases 3 that could cause NF-κB activation by IkBa binding (Li et al., 2015). Betaine treatment has also found a reduction in levels of high-mobility group protein expression and also in in mRNA that showed a restriction in inflammation. Additionally betaine is also able to cause reduction in the generation of endogenous damage-associated molecular pattern (DAMP) to cause inhibition of NF-κB pathway. So betaine may be suggested for its anti-inflammatory effects via inhibition of NF-κB signalling pathway (Zhang et al., 2013).

3.6 Betaine inhibits NLRP3 inflammasome activation

The pyrvin containing 3 (NLRP3) inflammasome (leucine rich family) has been known as a large cytoplasmic protein complex containing a domain for nucleotide-binding, adapter molecule ASC, NLRP3 a member of leucine rich repeat (NLR) family and mature caspase-1. When DAMPs are recognized by TLRs, NF-κB gets activated and the expression of mRNA is promoted in interleukin precursors
that include the pro-IL-1β and pro-IL-18 and also the NLRP3 (Sims and Smith, 2010). The NLRP3 when assembled completely, inflammasome brings about the activation of caspase-1 for mediating the mature IL-18 and IL-1β production. These are involved in the initiation of inflammation (Martinon et al., 2009).

3.7 Betaine regulates energy metabolism to relieve chronic inflammation

A number of chronic diseases like diabetes and obesity have been known to be the result of energy metabolism disorders and generally they are having systemic inflammation of low grade (Sik et al., 2014). Thus for inflammation mitigation, it is essential to restore the normal metabolism. Betaine has been reported for affecting the metabolism of glucose and lipids (Kathirvel et al., 2010; Li et al., 2017). In case of metabolism of lipids, there is excessive fat accumulation of fat as a result of the imbalance in lipid synthesis, oxidation and transportation. Various other factors that have been reported for such conditions are diets containing high fat, ethanol intake and antibiotics (Jung et al., 2013; Xu et al., 2015). Betaine has been found to restore the balance between fat synthesis and oxidation of fat for inhibiting the accumulation of fat (Xu et al., 2015; Song et al., 2007; Du et al., 2018). It has been found by Song and his colleagues that the increased activity of hepatic (AMP-activated protein kinase) may also be mechanistically involved (Song et al., 2007). AMPK plays the roles of regulator of a vital metabolic homeostasis and that of sensor of principal cellular energy. Various genes like acetyl CoA carboxylase, sterol regulatory element-binding protein-1c and fatty acid synthase are also controlled by AMPK. AMPK when activated is also known for inhibiting the synthesis of fatty acids and promoting the oxidation of fatty acids by regulating the concerned gene expression (Yang et al., 2017). Betaine has been found to increase the phosphorylation of AMPK and to inhibit the activity of acetyl CoA carboxylase as well as sterol regulatory element-binding protein-1c and the expression of fatty acid synthase (Song et al., 2007). All these results have been found to be supporting the finding of another studies carried out in diet-induced insulin-resistant mice in which AMPK could do the phosphorylation of SREBP-1c as well as SREBP-2 at Ser372 for inhibition of their activities so as to cause reduction in lipogenesis and accumulation of lipids (Li et al., 2011). Moreover, activated AMPK increases the uptake glucose by facilitating glucose transporter-GLUT-4 translocation, these outcomes have proved to be beneficial for insulin resistance (Russell et al., 1999). Talking about the mechanism of activation of AMPK, changes in the ratio of AMP:ATP in cells activates AMPK under normal conditions (Sang-Min, 2016). AMPK can also be activated independent of AMP: ATP ratio through adiponectin (Huypens et al., 2005; Bonnard et al., 2008). Another studies carried out by Song revealed that betaine is able for restoring the abnormal levels of adipokine NAFLD and it is also involved in the upregulation of adiponectin and down regulation of resistin and leptin in adipose cells for attenuating the dysregulation in metabolism of lipids. Betaine has also been supported for similar effects by in vitro studies carried by Olli in adipocytes (Olli et al., 2013). This study found that phosphorylation of AMPK has also been contributed by adiponectin upregulation (Wang et al., 2010). As these adipokines are involved in inflammation so this process of normalizing is anti-inflammatory (Olli et al., 2013). Besides activation of AMPK, betaine has been found to be influencing the other factors related to lipid metabolism potentially. As shown by earlier studies that betaine could cause reduction in the triglyceride accumulation of triglycerides in apolipoprotein B deficient mice by causing decrease in methylation of peroxisomal proliferator-activated receptor alpha (PPARα) methylation (Wang et al., 2013). Betaine has found to restrict the PPARγ transcriptional activity by the inhibition of binding of FOXO-1 to the PPARγ promoter for causing reduction in the accumulation of fat (Kim et al., 2016). It has been observed that besides PPARα, the upregulation of hepatic liver X receptor α (LXRα) was also found when the inhibition of oxidation of fatty acid was restored by betaine (Ge et al., 2016). Although betaine’s mechanism of activating the LXRα is not clear it may be said to be associated with an enzyme PRMT-3 related to SAM that could directly promote the activity of LXRα (Kim et al., 2015). Additionally, in a nephrotoxicity induced by cisplatin, betaine was found to inhibit peroxisidation of lipids by the suppression of activation of thiobarbituric acid reactive substance in kidneys that is mostly oxidative stress induced (Hagar et al., 2015). Betaine treatment is also able to ameliorate the transport of lipids. Betaine has found in maintaining the ratios of SAM:SAH in liver for enhancing the synthesis of phosphatidylcholines and normalizing the production of very-low-density lipoprotein (VLDL) by promoting the activity of PEMT (Kharbanda et al., 2009). Another study revealed that betaine has found to stimulate the expression of apoB gene for producing more of the VLDL (Huang et al., 1995). It has been demonstrated that with respect to the metabolism of glucose, insulin resistance has also found to be of inflammation related (Wellen and Hotamisligil, 2005; Barazzoni et al., 2018). It was discovered by Morgan et al that supplementation with betaine acted directly upon the insulin pathway for improving NAFLD (Kathirvel et al., 2010). Similar findings were also observed in another type 2 diabetes study (Kim et al., 2017).

3.8 Betaine mitigates ER Stress and Apoptosis

In endoplasmic reticulum (ER) stress, some proteins abnormally assembled in the lumen of endoplasmic reticulum. These abnormally assembled proteins may be either unfolded or misfolded (Schröder and Kaufman, 2005). Involvement of various other proteins has also been observed in ER stress, they are C/EBP homologous protein and GRP78 (glucose-regulated protein 78). They both are termed as the markers of ER stress (Zheng et al., 2014). The massive undesirable ER stress becomes the leading cause of the apoptosis of the cell. Apoptosis is programmed cell death and is also involved in the pathogenesis of inflammation and related diseases (Kondylis et al., 2017). Apoptosis involves both pathways i.e. extrinsic as well as intrinsic and the proteins of caspase family esp. caspase-3 finally complete the process (Larsen et al., 2010). Betaine is able to influence the pool of homocysteine directly and hyperhomocysteine has been reported for the induction of proteins that are misfolded, this ultimately results in ER stress (Ron, 2001). Cheng has reported in his studies that betaine stabilizes the levels of homocysteine and also causes the inhibition of the levels of CHOP and GRP78 and cell death as well (Ji et al., 2010). Similarly in another study, betaine was found to inhibit both CHOP and GRP78 and caused reduction in the activation of JNK (Wang et al., 2010). The JNK pathway is able to phosphorylate IRS-1 sites in multiple such as serine-
Mechanistically, ASC recruitment driven by NLR drives the in detail (Kanneganti, 2010; Kanneganti activated caspase-1 in the canonical inflammasome complex. IL-1β in vivo and in vitro studies and IL-1β been reported to suppress the activity of NF-kB and the expression of its downstream genes such as IL-1β reported to inhibit the production of IL-1β by the inhibition of MAP kinases (mitogen-activated protein kinase) and NIK/IKK (nuclear factor-including kinase/IkB kinase) in endothelial YPEN-1 cells in rats (Go Lee, 2013). Protein 38, JNK (c-Jun NH2-terminal kinase) and ERK1/2 (extracellular signal-regulated kinase) are the MAP kinases that are involved pro-inflammatory cytokines expressions (Kyriakis et al., 2012); and NIK/IKK leads to NF-kB activation by relieving IkB the inhibition (Jee et al., 2004).

Furthermore, betaine also causes the inhibition of TLRs that are involved in the activation of NF-κB. Betaine has been found to suppress the NF-kB activation in RAW264.7 cells stimulated by LPS (a TLR4 ligand) (Kim et al., 2014). In NAFLD rat models, Mechanistically, betaine has been found to inhibit the the expression of HMGB1 (high-mobility group box 1) and mRNA in hepatic tissues that regulates the TLR4 activation (Zhang et al., 2013). Betaine has been supposed to suppress the histone deacetylases 3 expression that binds IkBa for activate NF-kB activate in fructose-fed rat astrocytes (Li et al., 2015).

So summarizing the above findings, betaine has been demonstrated to dampen the activation of NF-kB in various in vivo and in vitro studies and IL-1β has been found to be most important NF-kB downstream gene. Betaine has been reputed to inhibit the production of IL-1β by inhibiting the NF-kB signaling pathway. The mechanism for the processing of IL-1β that has been identified most extensively is the activated caspase-1 in the canonical inflammasome complex. The canonical inflammasomes in details are found to contain caspase-1, cytosolic sensor molecules and adaptor molecule in detail (Kanneganti, 2010; Kanneganti et al., 2006). Mechanistically, ASC recruitment driven by NLR drives the activation of pro-caspase-1 that results in the cleavage of procaspase-1 and maturation of caspase-1. Then the pro-IL-1β is cleaved by caspase-1 for producing the IL-1β mature forms (Broz and Dixit, 2016; Prochnicki et al., 2016; Martinon et al., 2009).

3.10 Betaine in non-canonical inflammasome mediated processing of IL-1β

Caspases and modulators such as caspase-8/11 have been studied for their important roles in inflammasome-mediated maturation of IL-1β. Caspase-8 regulating the extrinsic pathway of apoptosis responsive to TNFR1 (TNF receptor 1) and activation of Fas (Oberst and Green, 2011) also been found to modulate the cleavage of pro-IL-1β exactly at the same site as that of caspase-1 (Maelfait et al., 2008; Gringhuis et al., 2012; Kang et al., 2013). Engagement of Fas has been found to trigger caspase-8-dependent production of IL-1β via completely independent pathway of caspase-1 in myeloid cells (Bosser et al., 2012). The caspase-1 and caspase-8 found to share the cleavage at same site, the caspase-8-dependent production of IL-1β does not need the participation of caspase-1. A caspase-8 inhibitor (CrmA) inhibits the LPS-induced IL-1β generation via an unclear mechanism (Maelfait et al., 2008). Moreover, another in vitro study reported that in RIP3−/− x Caspase-8−/− cells, both non-canonical and canonical activation of inflammasome and down-stream processing IL-1β are restrained extremely (Gurung et al., 2014).

In ER stress, caspase 8 mediated maturation of IL-1β is not requiring the expression of ASC (Shenderov et al., 2014). Caspase-8 is normally found to be present as an inactive enzyme in monomeric form under normal healthy conditions. But when FADD (Fas-associated death domain) binds to death receptors, the monomeric caspase-8 zymogens recruitment is facilitated that results in homodimerization caspase-8 and the subsequent activation of caspase-8 (Oberst and Green, 2005). Besides classically involved in apoptosis, caspase-8 has also been found to be vitally involved in the facilitating the signalling of NF-kB in B and T cells stimulated by antigens (Oberst and Green, 2005). In this way, caspase-8 and FADD control the NLRP3 inflammasome transcriptional priming by modulating the expression of pro-IL-1β and NLRP3. It has been demonstrated by increasing number of studies that betaine reduces the activity of caspase-8 and blocks the activation of caspase-8 significantly (Graaf et al., 2002; Garrett et al., 2013; Horio et al., 2001). Therefore, betaine may inhibit the production of IL-1β by the prevention of caspase-8 activity/activation induction. In dendritic cells (DCs) stimulated by mycobacterium or fungi, the dectin-1 stimulation can facilitate the Syk-dependent the CARD9-Bcl-10-MAL1T1 scaffold formation which in turn induces the activation of NF-kB and transcription IL-1β and also the aMAL1T1-caspase-8-ASC complex formation and activation which then mediates the pro-IL-1β processing (Gringhuis Dupaul-Chicoine and Saleh, 2012). As the caspase-8 activation is blocked by betaine, so it is suggested that betaine causes the reduction in the production of IL-1β via the suppression of the MAL1T1-caspase-8-ASC complex formation and activation. In summary, caspase-8 is importantly involved in controlling the differentiation of human macrophages (Buchrieser et al., 2018) and activation of human microglia and monocyte (Oliva-Martin et al., 2016; Venero et al., 2011). Caspase-8 obviously participates in cytokines production regulation. DCs and caspase-8-deficient
Betaine at pharmacological doses have been found to enhance the turnover of homocysteine (Kang, 1996; Obregon, 2003).

Betaine has also widely been studied for its antioxidant properties. It has been reported to relieve the oxidative pressure and thereby increasing the productivity of various crops, such as wheat, barley, sorghum, beans and soybean in stressful conditions (Bharwana et al., 2014; Osman, 2015).

It has been reported to modulate the IO and NS pathways positively by the attenuation of proinflammatory pathways that may be due to brain aging. It has been suggested by these evidences that betaine may be proved as an effective therapy for controlling the neurological disorders (Amiraslani et al., 2012).

3.13 Betaine against convulsions

Betaine has been reported for having the general anticonvulsant action as it has been studied for blocking the pentylenetetrazol and electroshock induced convulsions as effectively as it was observed to block the homocysteine induced convulsions. Although the mechanism for the same has not been known (Freed et al., 1979).

3.14 Role of betaine in performance and action

Aerobic endurance exercise

There is limited data regarding the investigation of betaine effects on aerobic endurance exercise and performance. Betaine has been observed to improve the ratio of NAD\(^+\) and NADH ratio by the nucleophilic H\(^+\) acceptance, yield of CH\(_4\) and DMG and by oxidizing the NADH to NAD\(^+\). In this way, it attenuates the cellular acidosis and improve the metabolism of glycolytes (Ghyczy and Boros 2001). The effects of betaine on a long running performance of competitive runners given with acute betaine supplementation in hot environmental conditions has been investigated by Armstrong et al the effects during mild dehydration. Subjects were dehydrated actively by 2.7 % body mass and after that they were given rehydration fluid, 1,000-mI, with or without betaine at 5 g per day. It was given 45 min prior to their performance trial that consisted of treadmill running for 75 min, running at 65 % VO\(_{2\text{peak}}\) and then running to exhaustion at 84 % VO\(_{2\text{peak}}\) following above. The plasma concentrations of betaine were increased with betaine supplementation, however, no variation was observed in sweat rate, loss of body mass, heart rate, skin or body temperature. Betaine was found to increase the consumption of plasma lactate and VO\(_2\) during the final stage. However, no statistically significance difference was observed in the time of sprint to exhaustion, duration of sprint was found to be increased. While lactate increase in plasma was not determinable whether this increased was due to improved clearance of lactate or due to increased glycolysis. Thus, the cellular state may have optimized by the betaine’s osmolytic effects by the increased osmolarity of cytoplasm and increased hydration of biopolymers, these effects ultimately result in an enhanced flux of glycolytes (Armstrong et al., 2008).

It has been shown by the epidemiologic studies that the elevated levels of homocysteine in serum (hyperhomocysteinemia) has been reported with the higher risks of stroke, cardiovascular disease, neurodegenerative diseases such as dementia, alzheimer disease, neural tube defects and many other neurological and metabolic neurological disorders (Craig, 2004). Betaine and folic acid at pharmacologic doses have been found to enhance the turnover of homocysteine (Kang, 1996; Obregon, 2003).
This may be attributed that the increased consumption of VO \(_2\) in the betaine supplemented group in the final stage of performance was due to enhanced stability of protein and increased consumption of oxygen by muscles. Trepanowski et al., has reported in 2 weeks study that in betaine supplemented group, muscle tissue was greatly saturated with oxygen after exercise in comparison with that in the placebo group. It has been studied that betaine protects the enzyme citrate synthase from thermodenaturation, therefore it may enhance the efficiency of Kreb’s cycle (Caldas et al., 1999). Summarizingly, betaine, due to having osmoprotective property may improve aerobic performance in high intensity fatigue causing exercises in challenging hot environments by prolonging the glycolytic flux and by enhancing the oxygen consumption by muscle tissue; however, this hypothesis needs to be evaluated by further research.

**Strength-based exercise**

It has been suggested that betaine also improves the performance in strength-based exercises (Craig, 2004), however, limited investigations are there for proving the same and the results are also observed to be conflicting. Improvement in fatigued squat repetitions has been demonstrated by Hoffman et al. but not been observed in vertical jump or bench press throw after betaine daily supplementation of 2.5 g for 15 days (Hoffman et al., 2009).

Hoffman et al. then conducted a follow-up study and reported that followed by the protocol of identical supplementation, improvements were not observed in mean force output, change in force output and isokinetic peak force output. On the other hand, Lee et al., after supplementation of betaine for 12 days has reported the improvements in production of force in isometric back squat, bench press, bench press throw power output and vertical jump power output. A trend for the improvement in vertical jump has been demonstrated by Cholewa et al. after supplementation with betaine for 6 weeks. In an another study by Pryor et al., betaine supplementation for 7 days has shown Pryor et al. increased in peak and mean power during four sprints of 12-s resisted cycle. Between testing sessions, the subjects in Lee et al. and Cholewa et al. unlike Hoffman et al. were given standardized resistance training. When power movements have not been the regular part of training, detections in power improvements are compromised so, there is a possibility that lacking of improvements in power of muscle strength can be attributed to the absence of training of power-specificity (Hoffman et al., 2011; Lee et al., 2010; Cholewa et al., 2013; Pryor et al., 2012).

Evidence has been provided by these findings that supplementation with betaine may improve muscular performance in protocols of high intensity resistance exercise. Contrary to this, no improvements with betaine treatment have been reported by Del Favero et al., in body composition, power output or strength. It is suggested by the authors that differences in dose, duration of supplementation and fitness status may be responsible for the outcomes (Frovaa et al., 2011). Improved muscular power and strength has been observed with 14 days of betaine supplementation by Apicella et al., 2012 and Hoffman et al., 2011. The testing of muscle power and sequence of testing in Del Strength was performed on third and sixth day respectively, this was followed by cessation of betaine supplementation and the testing of performance was performed on the final day of betaine supplementation. It was found that betaine is ergogenic, however, more research is needed for evaluating the adaptations sustainability after cessation of betaine supplementation (Apicella et al., 2012; Cholewa et al., 2013; Hoffman et al., 2009; Lee et al., 2010).

The conflicting results of betaine treatment were also seen on resistance-based work capacity. At 75 % and at 85 % 1 RM betaine was not found to improve single-set (Hoffman et al. 2009) and 3 sets of repetitions to fatigue respectively (Lee et al., 2010); however at 50% 1 RM, betaine supplementation for 14 days was found to increased volume and repetitions during 10 sets of bench press to fatigue (Trepanowski et al., 2011).

Therefore, betaine has been suggested to be having most ergogenic potential in high levels of metabolic stress generating exercise protocols, such protocols have high volumes and short periods of rest. Talking about the improvements in work capacity of upper body, betaine has not been observed to improve the capacity of work in free-weight back squats comparing to that of placebo (Cholewa et al., 2013). Abe et al. (2000), has found during a training protocol of twelve weeks that increased strength and muscle mass of upper body was at a greater magnitude as compared to that of lower body. So, there is a possibility that improvements in work capacity of muscles in lower body may require longer durations of study.

The mechanisms involved in improvement of performance by betaine have not been clearly identified. Metabolism of phosphagen at high rates is required during exercises of short duration and because betaine in diet increases the availability of serum SAM in healthy humans (Craig, 2004), betaine has been suggested by several researchers to improve the muscle performance by enhancing the synthesis of creatine (Hoffman et al., 2009, 2011; Lee et al., 2010; Trepanowski et al., 2011). Supplementation with betaine has been found to increase PCR in animal muscles (Wise et al., 1997); but it was observed that daily betaine supplementation of 2 g for 10 days had not cause any increase in PCR in muscles of humans (Del Favero et al., 2011). In the study conducted by Del Favero et al., the subjects were kept untrained and were not instructed for exercising, so there was minimal metabolism muscle PCR and thereby the additional SAM demand for creatine synthesis. Hence there is a possibility that the differences in the reported results by Del Favero et al., could be due to the reason that an exercise program was prescribed to the subjects that was relying on the phosphagen energy system, resulting in increased synthesis of creatine and therefore having the greater PCR content in muscle ultimately. Betaine may be ergogenic in its osmolytic effects also as during high-volume resistance training, enhanced glycolytic flux could be supported by an optimally hydrated cellular state. Metabolic stress has been found to result in the accumulation of inorganic ions and organic osmolytes including betaine (Haüssinger, 1996).

Accumulation of betaine in cells results in an increased osmolality of cytoplasm, hydration of biopolymer and cellular water redistribution. During stressful conditions, this helps to maintain the biochemical function (Brigotti et al., 2003). Due to the destabilization of function of enzymes, polarization and destabilized protein structure, and there is limited on influx (Petronini et al., 1992). Betaine has not
been found to affect protein structure or functions of enzymes and therefore is able to stabilize the functions of cellular metabolism osmotically stressful conditions (Dragolovich, 1994). Betaine has an important role in the enhancement of stability of proteins, so it has been named as a “counteracting” solute due to its ability to counter the denaturation of proteins by urea (Gilles, 1997). It has been shown that betaine also provides protection to heavy proteins of myosin chain and myosin ATPase against denaturation by urea (Ortiz-Costa et al., 2008) and also defends the muscle protein from dehydrogenation (Suarez et al., 2003). So, the ergogenic effects of betaine may be due to its ability to facilitate a hospitable environment in osmotically stressful conditions. Betaine has also found in defending citrate synthase from denaturation caused thermally (Caldas et al., 1999). It increases the capacity of work via facilitating recovery between sets by increasing the production of aerobic energy.

It was reported by Armstrong et al. (2008) that betaine was found to reduce thermal and thirst sensations during the final performance stages, and as reported by Hoffman et al. (2011) supplementation with betaine causes reduction in fatigue perceptions during high capacity exercising of upper body. Thermal conditions, thirst sensations and exertion perceptions, all influence the motivation and impact the body. Thermal conditions, thirst sensations and exertion perceptions, all influence the motivation and impact the performance (Knicker et al., 2011). Supplementation with phosphatidylcholine has been reported to improve focusing and reacting after intense exercise (Hoffman et al., 2010). As in PDC synthesis, SAM is donated by betaine (Stead et al., 2003). So, it may be suggested that betaine causes reduction in fatigue perceptions by increasing the content of free choline and a resultant increase in the synthesis of motor neuron acetylcholine.

4. Safety Concerns of Betaine

4.1 Toxicological studies

The Scientific Panel on Dietetic Products, Nutrition and Allergies (NDA) performed a number of in vitro and in vivo assays like (micronucleus assay after oral administration to mice, Salmonella reverse mutation assay and clastogenicity in human lymphocytes in vitro) to show that betaine was not genotoxic, for which they also given an substantial assessment report of available toxicity data (EFSA, 2005). At oral doses of 900 mg/kg b.w. per day, a reversible fatty hepatocytes vacuolisation was observed under the repeated toxicity studies in rats in one side or on the other side, a no observed adverse effect level (NOAEL) had not been identified at the lowest intake level. Further, the NDA were not arranged any reproduction and developmental toxicity studies. A scientific literature search was done from 2005-2103 (searching Medline32 and Toxnet33) by the FEEDAP Panel: while a lot of publications have been published regarding the physiological effects of betaine supplementation, e.g. on the metabolism of methionine or cholesterol, no appropriate information related to the unfavourable effects in ex-vivo animals has been published (EFSA, 2005).

4.2 Human studies

Apart from doing the toxicological studies on laboratory animals, the NDA Panel (EFSA, 2005) also evaluated many published clinical studies using betaine: while the results favoured the betaine because it was well tolerated, as the adverse effects was quite restricted as fully scrutinized by the NDA panel. Schwab et al., provided the better authentic study called a placebo-controlled randomised study (Schwab et al., 2002) performed on 42 obese subjects (22 in the treatment group and 20 in the control group) at a dose of 6 g/person/day for 18 weeks, given betaine or placebo. A reduction in homocysteine was observed, alongwith a minute lowering of diastolic blood pressure and uplifting of low-density lipoprotein (LDL) and total cholesterol in serum, in a 70 kg person at a dose of 90 mg/kg bw, while on most of the other parameters measured, it had no effect. From above, it was concluded that in susceptible people with such physiological and biochemical changes (e.g. with common problems regarding the control of blood pressure or cholesterol), it might have adverse effects, also this study could not give a tolerable upper intake level (UL).

5. Conclusion

In conclusion, this review has discussed the major pharmacological, physiological and the safety profile of betaine in various forms like an anti-inflammatory agents against many diseases, as osmotic agent against oxidative stress and a methyl group donor. These effects are essentially concerned with defending SAA metabolism from oxidative stress, by inhibiting NF-κB and NLRP3 inflammasome activity, energy metabolism regulation, and lessens endoplasmic reticulum stress and cell death. Although the animal experiment findings are engrossing, the clinical situation is completely opposite or much more complex than the actual one. Like many animal studies suggesting the effects of betaine as supplementation, but some clinical studies has shown divergent inferences. So, in order to reduce this contradiction, future studies will have to be focused on both pre-clinical and clinical experiments mutually, to prevent experimental errors and to confirm the medicinal role of betaine. As, we discussed about the antioxidant, anti-inflammatory effects, inhibition of proinflammation and its neuroprotective effects of betaine, it is very crucial, to further explore betaine for many diseases (Zha et al., 2018).

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