*Plant Archives* Vol. 19, Supplement 2, 2019 pp. 1021-1034 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

# 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 a an organis 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, neuroprotective effects, anti-Inflammatory effects, inhibition of NF-κB signaling pathway, inhibition of NLRP3 inflammasome activation, 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. Variouis 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 $(CH_3)_3N^+CH_2COO^-$ 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 a an organis osmolyte 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 (Akhavan-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*., 2008).

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*., 2005).

Therefore, 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, and secondly to study the safety concerns of betaine.

## 2. Pharmacokinetics of Betaine

As reported by various studies, betaine is absorbed (bacteria to vertebrates) as an osmoprotectant; betaine is absorbed through the duodenum (Kettunen *et al*., 2001; Kettunen *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 remaine 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 largly 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 previouis 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 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 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 dangerouse 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 nitric oxide synthase), COX-2 (cyclooxy-genase-2) and iNOS (inducible nitric 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-inducing kinase/IκB 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 deacety-lases 3 that could cause NF-κB activation by IκBα 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 pyrin 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-1 β 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 fascilitating 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 r *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 peroxidation 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 phosphatidylcholine 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 CHOP (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 an 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-

307 directly. The phosphorylation of insulin-stimulating IRS-1 tyrosine is prevented by these modifications leading to insulin resistance (Kathirvel *et al*., 2010). Betaine, in addition to inhibiting ER stress, also found to cause the inhibition of apoptosis. Gaur et al conducted a study on synovial fibroblasts in rheumatoid arthritis and found that betaine down regulates the ATF-3 (apoptosis transcription factor-3) molecule that is related to (Gaur *et al*., 2016). Additionally, betaine is also able to inhibit the proteins of caspase family. Kharbanda and his colleagues carried an in an *in vitro* study, in which adenosine addition to hepatocytes led to an increase the levels of SAH and increased activity of caspase-3 and betaine treatment was found to inhibit both of these (Kharbanda *et al*., 2005). The caspase-3 inhibition by betaine was also been observed in nephrotoxicity induced by cisplatin (Hagar *et al*., 2015). Moreover, betaine has also found to reduce the activity of caspase-3/7, caspase-8 and caspase-9 in epithelial cells of human cornea as well as the MDCK cells under the conditions of hyperosmosis significantly (Horio *et al*., 2001).

### 3.9 Betaine in canonical inflammasome-mediated processing of IL-1β

Immune cells such as macrophages and monocytes under unprovoked conditions either do not express or just express the IL-1β levels to the extreme low. But the triggers of proinflammation like IL-1a/6, TNF and TLR ligands induce the of NF-kB activation and the expression of IL-1β (Bauernfeind *et al*., 2009; Franchi *et al*., 2009). Betaine has been reported to suppress the activity of NF-kB and the expression of its downstream genes such as IL-1β through the inhibition of MAP kinases (mitogen-activated protein kinases) and NIK/IKK (nuclear factor-including kinase/IkB kinase) in endothelial YPEN-1 cells in rats (Go *et al*., 2005; Lee *et al*., 2013). Protein 38, JNK (c-Jun NH2-terminal kinase) and ERK1/2 (extracelluar 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- kB. Betaine has benn 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 repoted 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 adaptormolecule in detail (Kanneganti, 2010; Kanneganti *et al*., 2006). Mechanistically, ASC recruitment drived 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 studiedfor 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 (Bossaller *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 vitro study reported that in $RIP3^{-/-}$ × $Caspase\text{-}8^{-/-}$ 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-MALT1 scaffold formation which in turn induces the activation of NF-kB and transcription IL-1β and also the aMALT1-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 MALT1-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

macrophages are found to be hyperresponsive to the activation of TLR. IL-1β has found to be the marker of normal M1 macrophage and its polarization reuires caspase-8 (Cuda *et al*., 2016. The production of IL-1β has been observed to be inhibited by betaine and it appears to be caused by the reducing the activity of caspase-8. Although, the exact mechanisms for betaine in preventing the caspase-8 activation induction has not been reported so far (Buchrieser *et al*., 2018; Oliva-Martin *et al*., 2016).

### 3.11 Betaine against neurodegenerative diseases

Betaine has been reported for improving the memory impairment induced by lipopolysaccharide. It has been suggested that the protective effect of betaine on LPS-induced memory impairment is due to inhibition of neuroinflammation (Miwa *et al*., 2011). In an in vitro study carried out in PC12 cells, betaine has been proved be neuroprotective against the neurotoxicity induced by rotenone. In this study, betaine was found to attenuate the mitochondrial dysfunction, fragmentation of nuclear matter, depletion of ATP, depolarization of mitochondrial membrane depolarization, activation of caspase-3/7 and the production of ROS (reactive oxygen species), betaine was also found to decrease the levels of expression of caspase-9 and caspase-3. (Im *et al*., 2013). Betaine has also been evaluated by Masule *et al* for its neuroprotective effect in Sprague-Dawley rats using oxidative stress model induced by 6-OHDA in rat cerebellum. In this study, betaine was found to possess a potent antioxidant activity as compared to the standard, L-dopa+Benserazide group. Betaine was found to significantly reverse the IL-6 and IL-1β and TNF-α and has shown a dose dependent reduction in the levels of LPO and an increase in the activity of Catalase, GSH and SOD, GSH (Masule *et al*., 2019).

### 3.12 Physiological role of Betaine in body

Betaine is found naturally in various foods. The average daily betaine intake from diet may range from as low as 1 g and as high as 2.5 g depending upon its variable amounts present in different types of foods. As it is present in higher amounts in seafood and whole wheat (Hedemann *et al*., 2015; Corol *et al*., 2012).

The principle physiologic role of betaine is as an osmolyte and methyl donor (transmethylation). 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). 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 a an organis osmolyte and compatible solute which contributes to osmotic adjustment in animals, plants,bacteria, algae and fungi (Hedemann *et al*., 2015; Corol *et al*., 2012). So, betaine protects the proteins, enzymes and cells from environmental stress such as extreme temperature, high salinity and low water. Most effectively betaine has been studied as an osmolyte for the albumin hydration (Courtenay *et al.*, 2000), in the formation of water monolayer around proteins and maintaining the solvation of hemoglobin (Hundahl *et al.*, 2003).

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).

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 improvise 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-ml, 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 % $VO_{2PEAK}$ and then running to exhaustion at 84 % $VO_{2PEAK}$ 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).

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 reportedin 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 osmoprotection 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 (Frova *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 betain 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 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 (Ha¨ussinger, 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 dehydration (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 fascilitating 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 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*., 2006), 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 uplifiting 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 dieases (Zha *et al*., 2018).

## References

Abe, T.; DeHoyos, D.V.; Pollock, M.L. and Garzarella, L. (2000). Time course for strength and muscle thickness changes following upper and lower body resistance training in men and women. Eur. J Appl Physiol., 81:174–180.

Adamczak, B.; Wieczór, M.; Kogut, M.; Stangret, J. and Czub, J. (2016). Molecular basis of the osmolyte effect on protein stability: lesson from the mechanical unfolding of lysozyme. Biochem J., 473(20): 3705–24.

Ahn, M.; Kang, Y.; Moon, J.; Kim, S.; Moon, C. and Shin, T. (2014). Oral administration of betaine ameliorates ethanol-induced gastric injury in rats through its antioxidant effects. Orient. Pharm. Exp. Med., 14: 237–243.

Akhavan-Salamat, H. and Ghasemi, H.A. (2016). Alleviation of chronic heat stress in broilers by dietary supplementation of betaine and turmeric rhizome powder: dynamics of performance, leukocyte profile, humoral immunity, and antioxidant status. Trop. Anim. Health Prod., 48: 181–188.

Alirezaei, M (2004). Betaine protects cerebellum from oxidative stress following levodopa and benserazide administration in rats. IJBMS., 18: 950-957.

Alirezaei, M. (2014). Betaine as a methyl donor and an antioxidant agent in levodopa-induced hyperhomocysteinemia and oxidative stress in rat's kidney. Iran. J. Vet. Med., 8: 91–99.

Alirezaei, M.; Jelodar, G.; Ghayemi, Z. and Mehr, M.K. (2014). Antioxidant and methyl donor effects of betaine versus ethanol-induced oxidative stress in the rat liver. Comp. Clin. Pathol., 23: 161–168.

Alirezaei, M.; Gheisari, H.R.; Ranjbar, V.R. and Hajibemani, A. (2012). Betaine: a promising antioxidant agent for enhancement of broiler meat quality. Br. Poult. Sci., 53: 699–707.

Alirezaei, M.; Niknam, P. and Jelodar, G. (2012). Betaine elevates ovarian antioxidant enzyme activities and demonstrates methyl donor effect in non-pregnant rats. Int. J. Pept. Res. Ther., 18: 281–290.

Alirezaei, M.; Khoshdel, Z.; Dezfoulian, O.; Rashidipour, M. and Taghadosi, V. (2015). Beneficial antioxidant properties of betaine against oxidative stress mediated by levodopa/benserazide in the brain of rats. Journal of Physiology and Science., 65: 243.

Alirezaei, M., G. Jelodar, P. Niknam, Z. Ghayemi, S. Nazifi (2011). Betaine prevents ethanol-induced oxidative stress and reduces total homocysteine in the rat cerebellum. J PhysiolBiochem., 67(4):605-12.

Almashhadany, A.; Shackebaei, D.; Van der Touw, T.; Jones, G.L.; Suleiman, M.S. and King, N. (2015). Homocysteine exposure impairs myocardial resistance to ischaemia reperfusion and oxidative stress. Cell Physiol Biochem., 37(6): 2265–74.

Amiraslani, B.; Sabouni, F.; Abbasi, S.; Nazem, H. and Sabet, M. (2012). Recognition of Betaine as an Inhibitor of Lipopolysaccharide-Induced Nitric Oxide Production in Activated Microglial Cells. Iran Biomed J., 16(2): 84–89.

Apicella, J.M.; Lee, E.C.; Bailey, B.L. *et al.* (2012). Betaine supplementation enhances anabolic endocrine and Akt signaling in response to acute bouts of exercise. Eur J Appl Physiol., 113(3): 793-802.

Aravilli, R.K.; Vikram, S.L. and Kohila, V. (2017). Phytochemicals as potential antidotes for targeting NF-κB in rheumatoid arthritis. 3 Biotech., 7(4): 253.

Armstrong, L.E.; Casa, D.J.; Roti, M.W. *et al.* (2008). Influence of betaine consumption on strenuous running and sprinting in a hot environment. J Strength Cond Res., 22: 851.

Baggott, J.E. and Tamura, T. (2015). Homocysteine, iron and cardiovascular disease: a hypothesis. Nutrients., 7(2): 1108–18.

Barak, A.J.; Beckenhauer, H.C.; Tuma, D.J. (1996). Betaine, ethanol, and the liver: a review. Alcohol., 13(4): 395–8.

Barazzoni, R.; Gortan Cappellari, G.; Ragni, M. and Nisoli, E. (2018). Insulin resistance in obesity: an overview of fundamental alterations. Eat Weight Disord., 23(2):149–57.

Bauernfeind, F.G.; Horvath, G.; Stutz, A.; Alnemri, E.S.; MacDonald, K.; Speert, D. *et al.* (2009). Cutting edge, NF-kappaB activating pattern recognition and cytokine receptors license NLRP3 inflammasome activation by regulating NLRP3 expression. J Immunol., 183: 787–91.

Bharwana, S.A.; Ali, S.; Farooq, M.A.; Iqbal, N.; Hameed, A.; Abbas, F. and Ahmad, M.S.A. (2014). Glycine betaine-induced lead toxicity tolerance related to elevated photosynthesis, antioxidant enzymes suppressed lead uptake and oxidative stress in cotton. Turk. J. Bot., 38: 281–292.

Boisvert, F.M.; Côté, J.; Boulanger, M.C. and Richard, S. (2003). A proteomic analysis of argi-nine-methylated protein complexes. Mol Cell Proteomics., 2(12): 1319.

Bonnard, C.; Durand, A.; Vidal, H. and Rieusset, J. (2008). Changes in adiponectin, its recep-tors and AMPK activity in tissues of diet-induced diabetic mice. Diabetes Metab., 34(1): 52–61.

Borsook, M.E.; Billig, H.K. and Golseth, J.G. (1952). Betaine and glycocyamine in the treatment of disability resulting from acute anterior poliomyelitis. Ann West Med Surg., 6: 423–427.

Bossaller, L.; Chiang, P.I.; Schmidt-Lauber, C.; Ganesan, S.; Kaiser, W.J.; Rathinam, V.A. *et al.* (2012). Cutting edge: FAS (CD95) mediates noncanonical IL-1beta and IL- 18 maturation via caspase-8 in an RIP3-independent manner. J Immunol., 189: 5508–12.

Brigotti, M.; Petronini, P.G.; Carnicelli, D. *et al.* (2003). Effects of osmolarity, ions and compatible osmolytes on cell-free protein synthesis. Biochem J., 369: 369–374.

Broz, P. and Dixit, V.M. (2016). Inflammasomes: mechanism of assembly, regulation and signalling. Nat Rev Immunol., 16: 407–20.

Buchrieser, J.; Oliva-Martin, M.J.; Moore, M.D.; Long, J.C.D.; Cowley, S.A.; Perez-Simon, J.A. *et al.* (2018). RIPK1 is a critical modulator of both tonic and TLR responsive inflammatory and cell death pathways in human macrophage differentiation. Cell Death Dis., 9: 973.

Bucolo, C.; Fidilio, A.; Cbm, P.; Geraci, F.; Lazzara, F. and Drago, F. (2017). Antioxidant and osmoprotecting activity of taurine in dry eye models. Journal of Ocular Pharmacology & Therapeutics the Official Journal of the Association for Ocular Pharmacology & Therapeutics., 34(1-2): 188-194.

Caldas, T.; Demont-Caulet, N.; Ghazi, A. and Richarme, G. (1999). Thermoprotection by glycine betaine and choline. Microbiology., 145(Pt 9): 2543–2548.

Cholewa, J.M.; Guimarães-Ferreira, L. and Zanchi, N.E. (2014). Effects of betaine on performance and body composition: a review of recent findings and potential mechanisms. Amino Acids., 46(8):1785.

Cholewa, J.M.; Wyszczelska-Rokiel, M. and Glowacki, R. *et al.* (2013). Effects of betaine on body composition, performance, and homocysteine thiolactone. J Int Soc Sports Nutr., 10: 39.

Corol, D.I.; Ravel, C.; Raksegi, M.; Bedo, Z.; Charmet, G.; Beale, M.H.; Shewry, P.R. and Ward, J.L. (2012). Effects of genotype and environment on the contents of betaine, choline, and trigonelline in cereal grains. J. Agric. Food Chem., 60: 5471–5481.

Courtenay, E.S.; Capp, M.W.; Anderson, C.F. and Record, M.T. (2000). Vapor pressure osmometry studies of osmolyte-protein interactions: implications for the action of osmoprotectants in vivo and for the interpretation of "osmotic stress" experiments in vitro. Biochemistry., 39:4455–71.

Craig, S.A. (2004). Betaine in human nutrition. Am J Clin Nutr., 80(3): 539-549.

Craig, S.S., Craig, S.A.; Ganio, M.S.; Maresh, C.M.; Horrace, G.; Costa, K.A.D. and Zeisel, S.H. (2010). The betaine content of sweat from adolescent females. J Int Soc Sports Nutr., 7(1): 3.

Cuda, C.M.; Pope, R.M. and Perlman, H. (2016). The inflammatory role of phagocyte apoptotic pathways in rheumatic diseases. Nat Rev Rheumatol., 12:543–58.

Dasgupta, M. and Kishore, N. (2017). Selective inhibition of aggregation/fibrillation of bovine serum albumin by osmolytes: Mechanistic and energetics insights. Plos One., 12(2): e0172208.

Davies, K.J. (1995). Oxidative stress: the paradox of aerobic life. Biochem Soc Symp., 61: 1–31.

Davies, S.E.; Chalmers, R.A.; Randall, E.W. and Iles, R.A. (1988). Betaine metabolism in human neonates and developing rats. Clin Chim Acta., 178(3): 241–9.

Day, C.R. and Kempson, S.A. (2016). Betaine chemistry, roles, and potential use in liver disease. Biochim Biophys Acta., 1860(6):1098.

Del Favero, S.; Roschel, H.; Artioli, G. *et al.* (2011). Creatine but not betaine supplementation increases muscle phosphorylcreatine content and strength performance. Amino Acids., 42: 2299–2305.

Detopoulou, P.; Panagiotakos, D.B.; Antonopoulou, S.; Pitsavos, C. and Stefanadis, C. (2008). Dietary choline and betaine intakes in relation to concentrations of inflammatory markers in healthy adults: the ATTICA study. Am J Clin Nutr., 87: 424–430.

Dragolovich, J. (1994). Dealing with salt stress in animal cells: the role and regulation of glycine betaine concentrations. J Exp Zool., 268: 139–144.

Du, J.; Shen, L.; Tan, Z.; Zhang, P.; Zhao, X.; Xu, Y. *et al.* (2018). Betaine supplementation enhances lipid metabolism and improves insulin resistance in mice fed a high-fat diet. Nutrients., 10(2).

Dumaswala, U.J.; Zhuo, L.; Mahajan, S.; Nair, P.N.; Shertzer, H.G.; Dibello, P. *et al.* (2001). Glutathione protects chemokine-scavenging and antioxidative defense functions in human RBCs. Am J Physiol Cell Physiol., 280(4): C867.

Dupaul-Chicoine, J. and Saleh, M. (2012). A new path to IL-1beta production controlled by caspase-8. Nat Immunol., 13: 211–2.

EFSA (European Food Safety Authority), (2005). Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies (NDA) related to an application concerning the use of betaine as a novel food in the EU. The EFSA Journal., 191: 1-17.

Eklund, M.; Bauer, E.; Wamatu, J. and Mosenthin, R. (2005). Potential nutritional and physiological functions of betaine in livestock. Nutr Res Rev., 18: 31–48.

Franchi, L.; Eigenbrod, T. and Nunez, G. (2009). Cutting edge, TNF-alpha mediates sensitization to ATP and silica via the NLRP3 inflammasome in the absence of microbial stimulation. J Immunol., 183: 792–6.

Freed, W.J.; Gillin, J.C. and Wyatt, R.J. (1979). Anticonvulsant Properties of Betaine. Epilepsia., 20 (3): 209–213.

Freitas, I.; Boncompagni, E.; Tarantola, E.; Gruppi, C.; Bertone, V.; Ferrigno, A. *et al.* (2016). In situ evaluation of oxidative stress in rat fatty liver induced by a methionine- and choline-deficient diet. Oxid Med Cell Longev., 2016(3): 9307064.

Garrett, Q.; Khandekar, N.; Shih, S.; Flanagan, J.L.; Simmons, P.; Vehige, J. *et al.* (2013). Betaine stabilizes cell volume and protects against apoptosis in human corneal epithelial cells under hyperosmotic stress. Exp Eye Res., 108(3): 33–41.

Gaur, N.; Karouzakis, E.; Glück, S.; Bagdonas, E.; Jüngel, A.; Michel, B.A. *et al.* (2016). MicroRNAs interfere with DNA methylation in rheumatoid arthritis synovial fibroblasts. RMD Open., 2(2): e000299.

Ge, C.X.; Yu, R.; Xu, M.X.; Li, P.Q.; Fan, C.Y.; Li, J.M. *et al*. (2016). Betaine prevented fructose-induced NAFLD by regulating LXRα/PPARα pathway and allevi-ating ER stress in rats. Eur J Pharmacol., 770: 154–64.

Ghyczy, M. and Boros, M. (2001). Electrophilic methyl groups present in the diet ameliorate pathological states induced by reductive and oxidative stress: a hypothesis. Br J Nutr., 85: 409–414.

Gilles, R. (1997). Compensatory organic osmolytes in high osmolarity and dehydration stresses: history and perspectives. Comp Biochem Physiol., 117: 279–290.

Go, E.K., Jung, K.J.; Kim, J.M.; Lim, H.; Lim, H.K.; Yu, B.P. *et al*. (2007). Betaine modulates age-related NF-κB by thiol-enhancing action. Biol Pharm Bull., 30(12): 2244–9.

Go, E.K.; Jung, K.J.; Kim, J.Y.; Yu, B.P. and Chung, H.Y. (2005). Betaine suppresses proinflam-matory signaling during aging: the involvement of nuclear factor-kappaB via nuclear factor-inducing kinase/IkappaB kinase and mitogen-activated protein kinases. J Gerontol., 60(10): 1252.

Graf, D.; Kurz, A.K.; Reinehr, R.; Fischer, R.; Kircheis, G. and Haussinger, D. (2002). Prevention of bile acid-induced apoptosis by betaine in rat liver. Hepatology., 36(4 Pt 1):829–39.

Gringhuis, S.I.; Kaptein, T.M.; Wevers, B.A.; Theelen, B.; Van der Vlist, M.; Boekhout, T. *et al.* (2012). Dectin-1 is an extracellular pathogen sensor for the induction and processing of IL-1beta via a noncanonical caspase-8 inflammasome. Nat Immunol., 13: 246–54.

Gurung, P.; Anand, P.K.; Malireddi, R.K.; Vande Walle, L.; Van Opdenbosch, N.; Dillon, C.P. *et al.* (2014). FADD and caspase-8 mediate priming and activation of the canonical and noncanonical Nlrp3 inflammasomes. J Immunol., 192: 1835–46.

Ha¨ussinger, D. (1996). The role of cellular hydration in the regulation of cell function. Biochem J., 313(Pt 3): 697–710.

Hagar, H.; Medany, A.E.; Salam, R.; Medany, G.E. and Nayal, O.A. (2015). Betaine supple-mentation mitigates cisplatin-induced nephrotoxicity by abrogation of oxidative/nitrosative stress and suppression of inflammation and apoptosis in rats. Exp Toxicol Pathol., 67(2): 133–41.

Hedemann, M.S.; Theil, P.K.; Lærke, H.N. and Bach, K.E. (2015). Distinct difference in absorption pattern in pigs of betaine provided as a supplement or present naturally in cereal dietary fiber. J. Agric. Food Chem., 63: 2725−2733.

Hoffman, J.; Ratamess, N.; Kang, J. *et al.* (2006). Effect of creatine and beta-alanine supplementation on performance and endocrine responses in strength/power athletes. Int J Sport Nutr Exerc Metab., 16: 430–446.

Hoffman, J.R.; Ratamess, N.A.; Kang, J. *et al.* (2009). Effect of betaine supplementation on power performance and fatigue. J Int Soc Sports Nutr., 6: 7.

Hoffman, J.R.; Ratamess, N.A.; Kang, J. *et al.* (2011). Effect of 15 days of betaine ingestion on concentric and eccentric force outputs during isokinetic exercise. J Strength Cond Res., 25: 2235–2241.

Horio, M.; Ito, A.; Matsuoka, Y.; Moriyama, T.; Orita, Y.; Takenaka, M. and Imai, E. (2001). Apoptosis induced by hypertonicity in Madin Darley canine kidney cells: protective effect of betaine. Nephrol Dial Transplant., 16(3): 483–90.

Huang, L.S.; Voyiaziakis, E.; Markenson, D.F.; Sokol, K.A.; Hayek, T. and Breslow, J.L. (1995). Apo B gene knockout in mice results in embryonic lethality in homozygotes and neural tube defects, male infertility, and reduced HDL cholesterol ester and apo A-I transport rates in heterozygotes. J Clin Invest., 96(5): 2152–61.

Hundahl, C.; Fago, A.; Malte, H. and Weber, R.E. (2003). Allosteric effect of water in fish and human hemoglobins. J Biol. Chem., 278: 42769–73.

Huypens, P.; Moens, K.; Heimberg, H.; Ling, Z.; Pipeleers, D. and Van de Casteele, M. (2005). Adiponectin-mediated stimulation of AMP-activated protein kinase (AMPK) in pancreatic beta cells. Life Sci., 77(11): 1273–82.

Im, A.R.; Kim, Y.H.; Uddin, M.R.; Chae, S.; Lee, H.W.; Kim, Y.H.; Kim, Y.S. and Lee, M.Y. (2013). Betaine protects against rotenone-induced neurotoxicity in PC12 cells. Cell MolNeurobiol., 33(5): 625-35.

Je, J.H.; Lee, J.Y.; Jung, K.J.; Sung, B.; Go, E.K.; Yu, B.P. *et al*. (2004). NF-kappaB activation mechanism of 4-hydroxyhexenal via NIK/IKK and p38 MAPK pathway. FEBS Lett., 566(1–3): 183–9.

Ji, C. and Kaplowitz, N. (2003). Betaine decreases hyperhomocysteinemia, endoplasmic reticulum stress, and liver injury in alcohol-fed mice. Gastroenterology., 124(5):1488.

Ji, C.; Shinohara, M.; Kuhlenkamp, J.; Chan, C. and Kaplowitz, N. (2010). Mechanisms of protection by the betaine-homocysteine methyltransferase/betaine system in HepG2 cells and primary mouse hepatocytes. Hepatology., 46(5): 1586–96.

Jin, P.; Zhang, Y.; Shan, T.; Huang, Y.; Xu, J. and Zheng, Y. (2015). Low temperature conditioning alleviates chilling injury in loquat fruit and regulates glycine betaine content and energy status. J. Agric. Food Chem., 63: 3654−3659.

Jin, Z.; Mendu, S.K. and Birnir, B. (2013). GABA is an effective immunomodulatory mole-cule. Amino Acids., 45(1): 87–94.

Jung, Y.S.; Kwak, H.E.; Choi, K.H. and Kim, Y.C. (2003). Effect of acute ethanol administration on S-amino acid metabolism: increased utilization of cysteine for synthesis of taurine rather than glutathione. Adv Exp Med Biol., 526:245.

Jung, Y.S.; Sun, J.K.; Kwon, D.Y.; Ahn, C.W.; Kim, Y.S.; Choi, D.W. *et al.* (2013). Alleviation of alcoholic liver injury by betaine involves an enhancement of antioxidant defense via regulation of sulfur amino acid metabolism. Food Chem Toxicol., 62(12): 292–8.

Jung, Y.S.; Sun, J.K.; Kwon, D.Y.; Ahn, C.W.; Kim, Y.S.; Choi, D.W. *et al*. (2013). Alleviation of alcoholic liver injury by betaine involves an enhancement of antioxidant defense via regulation of sulfur amino acid metabolism. Food Chem Toxicol., 62(12):292–8.

Kang, S.S. (1996). Treatment of hyperhomocyst(e)inemia: physiological basis. J Nutr., 126:1273S–5S.

Kang, T.B.; Yang, S.H.; Toth, B.; Kovalenko, A. and Wallach, D. (2013). Caspase-8 blocks kinase RIPK3-mediated activation of the NLRP3 inflammasome. Immunity., 38: 27–40.

Kanneganti, T.D (2010). Central roles of NLRs and inflammasomes in viral infection. Nat Rev Immunol., 10: 688–98.

Kanneganti, T.D.; Ozoren, N.; Body-Malapel, M.; Amer, A.; Park, J.H.; Franchi, L. *et al.* (2006). Bacterial RNA and small antiviral compounds activate caspase-1 through cryopyrin/Nalp3. Nature. 440: 233–6.

Kathirvel, E.; Morgan, K.; Nandgiri, G.; Sandoval, B.C.; Caudill, M.A.; Bottiglieri, T. *et al.* (2010). Betaine improves nonalcoholic fatty liver and associated hepatic insulin resistance: a potential mechanism for hepatoprotection by betaine. Am J Physiol Gastrointest Liver Physiol., 299(5): G1068.

Kempson, S.A.; Zhou, Y. and Danbolt, N.C. (2014). The betaine/GABA transporter and betaine: roles in brain, kidney, and liver. Front Physiol., 5(5):159.

Kettunen, H.; Tiihonen, K.; Peuranen, S.; Saarinen, M.T. and Remus, J.C. (2001). Dietary beta-ine accumulates in the liver and intestinal tissue and stabilizes the intestinal epithelial structure in healthy and coccidia-infected broiler chicks. Comp Biochem Physiol A Mol Integr Physiol., 130(4):759–69.

Kettunen, H.; Peuranen, S.; Tiihonen, K. and Saarinen, M. (2001). Intestinal uptake of betaine in vitro and the distribution of methyl groups from betaine, choline, and methionine in the body of broiler chicks. Comp Biochem Physiol A Mol Integr Physiol., 128(2): 269.

Kharbanda, K.K.; Rogers, D.D.; Mailliard, M.E.; Siford, G.L.; Barak, A.J.; Beckenhauer, H.C. *et al.* (2005). Role of elevated S-adenosylhomocysteine in rat hepatocyte apoptosis: protection by betaine. Biochem Pharmacol., 70(12): 1883–90.

Kharbanda, K.K.; Todero, S.L.; Ward, B.W. and Tuma, D.J. (2009). Betaine administration corrects ethanol-induced defective VLDL secretion. Mol Cell Biochem., 327(1–2):75–8.

Kim, D.H.; Lee, B.; Min, H.P.; Min, J.K.; An, H.J.; Lee, E.K. *et al.* (2016). Molecular mecha-nism of betaine on hepatic lipid metabolism: inhibition of FoxO1 binding to PPARg. J Agric Food Chem., 64(36):6819.

Kim, D.H.; Sung, B.; Kang, Y.J.; Jang, J.Y.; Hwang, S.Y.; Lee, Y. *et al*. (2014). Anti-inflammatory effects of betaine on AOM/DSS-induced colon tumorigenesis in ICR male mice. Int J Oncol., 45(3):1250.

Kim, D.H.; Sung, B.; Kang, Y.J.; Jang, J.Y.; Hwang, S.Y.; Lee, Y. *et al.* (2014). Anti-inflammatory effects of betaine on AOM/DSSinduced colon tumorigenesis in ICR male mice. Int J Oncol., 45:1250–6.

Kim, D.H.; Kim, S.M.; Lee, B.; Lee, E.K.; Chung, K.W.; Moon, K.M. *et al*. (2017). Effect of betaine on hepatic insulin resistance through FOXO1-induced NLRP3 inflammasome. J Nutr Biochem., 45:104.

Kim, D.I.; Park, M.J.; Lim, S.K.; Park, J.I.; Yoon, K.C.; Han, H.J. *et al.* (2015). PRMT3 regulates hepatic lipogenesis

through direct interaction with LXRα. Diabetes., 64(1): 60.

Knicker, A.J.; Renshaw, I.; Oldham, A.R.H. and Cairns, S.P. (2011). Interactive processes link the multiple symptoms of fatigue in sport competition. Sport Med., 41: 307–328.

Knight, L.S.; Piibe, Q.; Lambie, I.; Perkins, C. and Yancey, P.H. (2017). Betaine in the brain: characterization of betaine uptake, its influence on other osmolytes and its potential role in neuroprotection from osmotic stress. Neurochem Res., 42(12): 3490–503.

Kondylis, V.; Kumari, S.; Vlantis, K. and Pasparakis, M. (2017). The interplay of IKK, NF-κB and RIPK1 signaling in the regulation of cell death, tissue homeostasis and inflammation. Immunol Rev., 277(1): 113.

Konstantinova, S.V.; Tell, G.S.; Vollset, S.E.; Nygård, O.; Bleie, Ø. and Ueland, P.M. (2008). Divergent associations of plasma choline and betaine with components of metabolic syndrome in middle age and elderly men and women. J Nutr., 138:914–920.

Kramer, K.; Harrington, E.O.; Lu, Q.; Bellas, R.; Newton, J. and Sheahan, K.L. *et al.* (2003). Isoprenylcysteine carboxyl methyltransferase activity modulates endothelial cell apoptosis. Mol Biol Cell., 14(3): 848.

Kyriakis, J.M. and Avruch, J. (2012). Mammalian MAPK signal transduction pathways activated by stress and inflammation: a 10-year update. Physiol Rev., 92: 689–737.

Larsen, B.D.; Rampalli, S.; Burns, L.E.; Brunette, S.; Dilworth, F.J. and Megeney, L.A. (2010). Caspase 3/caspase-activated DNase promote cell differentiation by induc-ing DNA strand breaks. Proc Natl Acad Sci U S A., 107(9): 4230–5.

Lawsonyuen, A. and Levy, H.L. (2006). The use of betaine in the treatment of elevated homocysteine. Mol Genet Metab., 88(3): 201–7.

Lee, E.C.; Maresh, C.M.; Kraemer, W.J. *et al.* (2010). Ergogenic effects of betaine supplementation on strength and power performance. J Int Soc Sports Nutr., 7:27.

Lee, E.K.; Jang, E.J.; Jung, K.J.; Kim, D.H.; Yu, B.P. and Chung, H.Y. (2013). Betaine attenuates lysophosphatidylcholine-mediated adhesion molecules in aged rat aorta: modulation of the nuclear factor-kappaB pathway. Exp Gerontol., 48: 517–24.

Lever, M., P.C. Sizeland, C.M. Frampton and S.T. Chambers (2004). Short and long-term variation of plasma glycine betaine concentrations in humans. Clin Biochem., 37(3):184–90.

Li, J.M.; Ge, C.X.; Xu, M.X.; Wang, W.; Yu, R.; Fan, C.Y. *et al.* (2015). Betaine recovers hypothalamic neural injury by inhibiting astrogliosis and inflammation in fructose-fed rats. Mol Nutr Food Res., 59(2): 189–202.

Li, S.; Wang, H.; Wang, X.; Wang, Y. and Feng, J. (2017). Betaine affects muscle lipid metabo-lism via regulating the fatty acid uptake and oxidation in finishing pig. J Anim Sci Biotechnol., 8(1): 72.

Li, Y.; Xu, S.; Mihaylova, M.; Zheng, B.; Hou, X.; Jiang, B. *et al.* (2011). AMPK phosphory-lates and inhibits SREBP activity to attenuate hepatic steatosis and atheroscle-rosis in diet-induced insulin resistant mice. Cell Metab., 13(4): 376–88.

Maelfait, J.; Vercammen, E.; Janssens, S.; Schotte, P.; Haegman, M.; Magez, S. *et al.* (2008). Stimulation of Toll-like receptor 3 and 4 induces interleukin- 1beta maturation by caspase-8. J Exp Med., 205: 1967–73.

Martínez, Y.; Li, X.; Liu, G.; Bin, P.; Yan, W.; Más, D. *et al.* (2017). The role of methionine on metabolism, oxidative stress, and diseases. Amino Acids., 49(12): 2091–8.

Martinon, F.; Mayor, A. and Tschopp, J. (2009). The inflammasomes: guardians of the body. Annu Rev Immunol., 27(27): 229.

Masule, M.V.; Shinde, S.D.; Kurkute, S.S. and Salve, B.U. (2019). Evaluation of Antioxidant and Anti Parkinsonism Activity of Betaine in Experimental Rats. Journal of Drug Delivery & Therapeutics., 9(2-s): 427-421.

Mcneil, S.D.; Nuccio, M.L. and Hanson, A.D. (1999). Betaines and related osmoprotectants. Targets for metabolic engineering of stress resistance. Plant Physiol., 120(4): 945–9.

Meng, B.; Gao, W.; Wei, J.; Yang, J.; Wu, J.; Pu, L.; *et al.* (2013). Quercetin reduces serum homocysteine level in rats fed a methionine-enriched diet. Nutrition., 29(4): 661–6.

Miwa, M.; Tsuboi, M.; Noguchi, Y.; Enokishima, A.; Nabeshima, T. and Hiramatsu, M. (2011). Effects of betaine on lipopolysaccharide-induced memory impairment in mice and the involvement of GABA transporter 2. J Neuroinflammation., 8: 153.

Monaco, C.; Andreakos, E.; Kiriakidis, S.; Mauri, C.; Bicknell, C.; Foxwell, B. *et al.* (2004). Canonical pathway of nuclear factor kappa B activation selectively regulates proinflammatory and prothrombotic responses in human atherosclerosis. Proc Natl Acad Sci U S A., 101(15): 5634–9.

Mosharov, E.; Cranford, M.R. and Banerjee, R. (2000). The quantitatively important relationship between homocysteine metabolism and glutathione synthesis by the transsulfuration pathway and its regulation by redox changes. Biochemistry., 39(42): 13005–11.

Neuhofer, W. and Beck, F.X. (2005). Cell survival in the hostile environment of the renal medulla. Annu Rev Physiol., 67(67): 531–55.

Nishimakimogami, T.; Yao, Z. and Fujimori, K. (2002). Inhibition of phosphatidylcholine synthesis via the phosphatidylethanolamine methylation pathway impairs incorporation of bulk lipids into VLDL in cultured rat hepatocytes. J Lipid Res., 43(7): 1035–45.

Oberst, A. and Green, D.R. (2011). It cuts both ways: reconciling the dual roles of caspase 8 in cell death and survival. Nat Rev Mol Cell Biol., 12: 757–63.

Obregon, D.F.; Murthy, S.N.; McNamara, D.B. and Fonseca, V.A. (2003). Novel approaches to the treatment of hyperhomocysteinaemia. Expert OpinTher Patents., 13: 1023–35.

Oliva-Martin, M.J.; Sanchez-Abarca, L.I.; Rodhe, J.; Carrillo-Jimenez, A.; Vlachos, P.; Herrera, A.J. *et al.* (2016). Caspase-8 inhibition represses initial human monocyte activation in septic shock model. Oncotarget., 7: 37456–70.

Olli, K.; Lahtinen, S.; Rautonen, N. and Tiihonen, K. (2013). Betaine reduces the expression of inflammatory adipokines caused by hypoxia in human adipocytes. Br J Nutr., 109(1):43–9.

Ortiz-Costa, S.; Sorenson, M.M. and Sola-Penna, M. (2008). Betaine protects urea-induced denaturation of myosin subfragment-1. FEBS J., 275:3388–3396.

Osman, H.S. (2015). Enhancing antioxidant yield relationship of pea plant under drought at different growth stages by exogenously applied glycine betaine and proline. Ann. Agric. Sci., 60: 389–402.

Petronini, P.G.; De Angelis, E.M.; Borghetti, P. *et al.* (1992). Modulation by betaine of cellular responses to osmotic stress. Biochem J., 282:69–73.

Prochnicki, T.; Mangan, M.S. and Latz, E. (2016). Recent insights into the molecular mechanisms of the NLRP3 inflammasome activation. F1000Res., 5:F1000.

Pryor, J.L.; Craig, S.A. and Swensen, T. (2012). Effect of betaine supplementation on cycling sprint performance. J Int Soc Sports Nutr., 9: 12.

Pummer, S.; Dantzler, W.H.; Lien, Y-HH.; Moeckel, G.W.; Völker, K. and Silbernagl, S. (2000). Reabsorption of betaine in Henle's loops of rat kidney in vivo. Am J Physiol - Renal Physiol., 278: F434-F439.

Rando, R.R. (1996). Chemical biology of isoprenylation/methylation. Biochem Soc Trans., 24(3): 682.

Ratriyanto, A.; Mosenthin, R.; Bauera, E. and Eklund, M. (2009). Metabolic, osmoregulatory and nutritional functions of betaine in monogastric animals. Asian-australas J Anim Sci., 22(10):1461–76.

RC, P.; Swarup, D. and Dwivedi, S.K. (2001). Antioxidant effects of alpha tocopherol, ascorbic acid and l-methionine on lead induced oxidative stress to the liver, kidney and brain in rats. Toxicology., 162(2): 81.

Ron, D (2001). Hyperhomocysteinemia and function of the endoplasmic reticulum. J Clin Invest., 107(10): 1221–2.

Russell, R.R.; Bergeron, R.; Shulman, G.I.; Young, L.H. (1999). Translocation of myo-cardial GLUT-4 and increased glucose uptake through activation of AMPK by AICAR. Am J Physiol., 277(2): 643–9.

Sang-Min, J. (2016). Regulation and function of AMPK in physiology and diseases. Exp Mol Med., 48(7): e245.

Schröder, M. and Kaufman, R.J. (2005). ER stress and the unfolded protein response. Mutat Res., 569(1): 29–63.

Schuliga, M. (2015). NF-kappaB signaling in chronic inflammatory airway disease. Biomolecules., 5(3): 1266–83.

Schwab, U.; Torronen, A.; Toppinen, L.; Alfthan, G.; Saarinen, M.; Aro, A. and Uusitupa, M. (2002). Betaine supplementation decreases plasma homocysteine concentrations but does not affect body weight, body composition, or resting energy expenditure in human subjects. American Journal of Clinical Nutrition., 76: 961–967.

Schwahn, B.C.; Hafner, D.; Hohlfeld, T.; Balkenhol, N.; Laryea, M.D.; Wendel, U. (2003). Pharmacokinetics of oral betaine in healthy subjects and patients with homocystinuria. British Journal of Clinical pharmacology., 55(1): 6–13.

Shenderov, K.; Riteau, N.; Yip, R.; Mayer-Barber, K.D.; Oland, S.; Hieny, S. *et al.* (2014). Cutting edge, Endoplasmic reticulum stress licenses macrophages to produce mature IL-1beta in response to TLR4 stimulation through a caspase-8- and TRIF-dependent pathway. J Immunol., 192: 2029–33.

Shinohara, M. and Ji, C.N. (2010). Differences in betaine-homocysteine methyltransferase expression, endoplasmic reticulum stress response, and liver injury between alcohol-fed mice and rats. Hepatology., 51(3): 796.

Sies, H. (1997). Oxidative stress: oxidants and antioxidants. Exp Physiol., 82(2): 291–5.

Sik, C.S.; Young, H.J.; Jae, H.I.; In, K.J. and Bum, K.J. (2016). Adipose tissue remodeling: its role in energy metabolism and metabolic disorders. Front Endocrinol., 7:30.

Sims, J.E. and Smith, D.E. (2010). The IL-1 family: regulators of immunity. Nat Rev Immunol., 10(2): 89.

Song, Z.; Deaciuc, I.; Zhou, Z.; Song, M.; Chen, T.; Hill, D. *et al.* (2007). Involvement of AMP-activated protein kinase in beneficial effects of betaine on high-sucrose diet-induced hepatic steatosis. Am J Physiol Gastrointest Liver Physiol., 293(4): G894.

Stead, L.M.; Brosnan, J.T.; Brosnan, M.E. *et al*. (2006). Is it time to reevaluate methyl balance in humans? Am J Clin Nutr., 83: 5–10.

Suarez, M.C.; Machado, C.J.V.; Lima, L.M.T.R. *et al.* (2003). Role of hydration in the closed-to-open transition involved in $Ca^{2+}$ binding by troponin C. Biochemistry., 42: 5522–5530.

Tan, D.X.; Manchester, L.C.; Terron, M.P.; Flores, L.J. and Reiter, R.J. (2007). One molecule, many derivatives: a never-ending interaction of melatonin with reactive oxygen and nitrogen species? J Pineal Res., 42(1):28–42.

Trepanowski, J.F.; Farney, T.M.; McCarthy, C.G. *et al.* (2011). The effects of chronic betaine supplementation on exercise performance, skeletal muscle oxygen saturation and associated biochemical parameters in resistance trained men. J Strength Cond Res., 1: 11.

Tsai, M.C. and Huang, T.L. (2015). Increased activities of both superoxide dismutase and catalase were indicators of acute depressive episodes in patients with major depressive disorder. Psychiatry Research., 235: 38–42.

Ueland, P.M. and Refsum, H. (1989). Plasma homocysteine, a risk factor for vascular disease: plasma levels in health, disease, and drug therapy. J Lab Clin Med., 114(5):473–501.

Veis, J.H.; Molitoris, B.A.; Teitelbaum, I.; Mansour, J.A. and Berl, T. (1991). Myo-inositol uptake by rat cultured inner medullary collecting tubule cells: effect of osmolality. Am J Physiol., 260(5): F619.

Venero, J.L.; Burguillos, M.A.; Brundin, P. and Joseph, B. (2011). The executioners sing a new song: killer caspases activate microglia. Cell Death Differ., 18: 1679–91.

Wang, L.; Chen, L.; Tan, Y.; Wei, J.; Chang, Y.; Jin, T. *et al.* (2013). Betaine supplement alleviates hepatic triglyceride accumulation of apolipoprotein E deficient mice via reducing methylation of peroxisomal proliferator-activated receptor alpha promoter. Lipids Health Dis., 12(1):34.

Wang, Z.; Yao, T.; Pini, M.; Zhou, Z.; Fantuzzi, G. and Song, Z. (2010). Betaine improved adipose tissue function in mice fed a high-fat diet: a mechanism for hepatoprotective effect of betaine in nonalcoholic fatty liver disease. Am J Physiol Gastrointest Liver Physiol., 298(5):G634.

Warren, L.K.; Lawrence, L.M. and Thompson, K.N. (1999). The influence of betaine on untrained and trained horses exercising to fatigue. J Anim Sci., 77: 677–684.

Wellen, K.E. and Hotamisligil, G.S. (2005). Inflammation, stress, and diabetes. J Clin Invest., 115(5): 1111–9.

Williams, K.T. and Schalinske, K.L. (2007). New insights into the regulation of methyl group and homocysteine metabolism. J Nutr., 137(2): 311–4.

Wise, C.K.; Cooney, C.A.; Ali, S.F. and Poirier, L.A. (1997). Measuring Sadenosylmethionine in whole blood, red blood cells and cultured cells using a fast preparation method and high-performance liquid chromatography. J Chromatogr., 696:145–152.

Xu, L.; Huang, D.; Hu, Q.; Wu, J.; Wang, Y. and Feng, J. (2015). Betaine alleviates hepatic lipid accumulation via enhancing hepatic lipid export and fatty acid oxidation in rats fed with a high-fat diet. Br J Nutr., 113(12):1835–43.

Yamamoto, A.; Takagi, H.; Kitamura, D.; Tatsuoka, H.; Nakano, H.; Kawano, H. *et al*. (1998). Deficiency in protein l-isoaspartyl methyltransferase results in a fatal progressive epilepsy. J Neurosci., 18(6):2063–74.

Yancey, P.H. (2005). Organic osmolytes as compatible, metabolic and counteracting cytoprotectants in high osmolarity and other stresses. J Exp Biol., 208(15):2819–30.

Yang, Z.; Hu, X.; Liu, Y.; Dong, S.; Wen, Z.; He, W. *et al.* (2017). ROS signaling under metabolic stress: cross-talk between AMPK and AKT pathway. Mol Cancer., 16(1): 79.

Yi, E.Y. and Kim, Y.J. (2012). Betaine inhibits in vitro and in vivo angiogenesis through suppression of the NF-kappaB and Akt signaling pathways. Int J Oncol., 41(5):1879–85.

Zeisel, S. (2017). Choline, other methyl-donors and epigenetics. Nutrients., 9(5): 445.

Zeisel, S.H. (2013). Metabolic crosstalk between choline/1-carbon metabolism and energy homeostasis. Clin Chem Lab Med., 51(3): 467–75.

Zeisel, S.H.; Mar, M.H.; Howe, J.C. and Holden, J.M. (2003). Concentrations of choline-containing compounds and betaine in common foods. J Nutr., 133(5):1302–7.

Zha, G.; He, F.; Wu, C.; Li, P.; Li, N.; Deng, J.; Zhu, G.; Ren, W. and Peng, Y. (2018). Betaine in inflammation: Mechanistic Aspects and Applications. Front. Immunol., 9:1070.

Zhang, B.; Dong, J.L.; Chen, Y.L.; Liu, Y.; Huang, S.S.; Zhong, X.L. *et al*. (2017). Nrf2 mediates the protective effects of homocysteine by increasing the levels of GSH content in HepG2 cells. Mol Med Rep.,16(1): 597.

Zhang, M.; Wu, X.; Lai, F.; Zhang, X.; Wu, H. and Min, T. (2016). Betaine inhibits hepatitis B virus with an advantage of decreasing resistance to lamivudine and interferon α. J. Agric. Food Chem., 64(20): 4068-77.

Zhang, W.; Wang, L.W.; Wang, L.K.; Li, X.; Zhang, H.; Luo, L.P. *et al.* (2013). Betaine protects against high-fat-diet-induced liver injury by inhibition of highmobility group box 1 and Toll-like receptor 4 expression in rats. Dig Dis Sci., 58(11):3198–206.

Zheng, Y.Z.; Cao, Z.G.; Hu, X.; and Shao, Z.M. (2014). The endoplasmic reticulum stress markers GRP78 and CHOP predict disease-free survival and responsive-ness to chemotherapy in breast cancer. Breast Cancer Res Treat., 145(2): 349–58.