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"The protective effect of dietary buckwheat under the stress caused by bisphenol A"

A Thesis Submitted to Siberian Federal University Krasnoyarsk, Russian Federation in partial fulfillment of the requirements for the degree of Master of Science Under supervision of Dr. Shubhra Pande

ABSTRACT

During decades plastics are used for a wide variety of commercial applications all over the world, however, plastics and its products have been associated to a number of health risks due to harmful components. Bisphenol A(BPA) is a synthetic compound that is added to many commercial plastic products, including food containers and hygiene products, and many other items. BPA-containing plastics are subject to degradation, resulting in the ingestion of BPA from many different sources when served hot. BPA has been shown to cause detrimental effects on health, even small concentrations. It may disrupt the cells function due to its ability to act as an estrogen mimic. Along with being implicated in the formation of cancers and the metabolic syndrome, BPA has also been linked to inducing oxidative stress, which can lead to damaged DNA, promote tumorigenesis, and eventually causes cell death. Because of its pro-oxidizing role, it was hypothesized that BPA could increase the likelihood of cells becoming senescent and can cause liver, kidneys, brain, and other organs injury by forming reactive oxygen species. Daily nutrition enriched antioxidants can prevent DNA damage and can enhance the activity of genes called sirtuins. The seven-enzyme sirtuin family is able to control many cell functions, including histone deacetylation, protein acylation, and deacetylation. This group of enzymes plays an important role in regulating oxidative stress-related processes and functions, including longevity, mitochondrial function, DNA damage repair, and metabolism. Buckwheat has become nutrient source due to its high mineral and antioxidant content. Its benefits may include improved oxidative stress control. Two types of buckwheat, common buckwheat (*Fagopyrum esculentum*) and Tartary buckwheat (*Fagopyrum tartaricum*), are most widely grown for nutrition. Tartary buckwheat has a higher antioxidant content than common buckwheat. It includes rutin, quercetin, vitexin, D-chiro-inositol.

This study aimed to evaluate whether exposure to BPA induce oxidative stress, liver damage, affecting oxidant/antioxidant balance in the liver and stomach of albinos Wistar male rats.

KEY WORDS: Bisphenol A, oxidative stress, DNA damage, sirtuin genes, buckwheat, antioxidants.

CHAPTER I

INTRODUCTION

Plastics are used consistently due to their durability, wide application, ease of use and low cost. However, the overuse of these materials came with unexpected problems. Plasticizers such as bisphenol A have been found to cause endocrine disorders, resulting in reproductive diseases and cancer in humans, and even feminization in aquatic species. In recent years, Bisphenol A has been causing various problems related to metabolic syndrome, diabetes, obesity and heart disease. This chemical is a cell contaminant that is toxic and mimic estrogen in mammalian cells.

Bisphenol A (BPA) is a chemical that is widely used as a commercial plasticizer in the production of polycarbonate plastics. BPA was invented by the reaction of phenol with acetone by the Russian chemist Aleksandr Dianin in 1891 but it's synthesis was first reported by Theodor Zincke in Germany in 1905 (Allard, 2014). Scientists discovered evidence of BPA toxicity in 1930. Between 1940's and 1950's the chemical industry begins to use BPA to manufacture a hard plastic called polycarbonate, and to make epoxy resins 1940 and 1950 years**.** (Jane Houlihan, 2008)**.** Originally, it was used as an epoxy resin to give primers and protective coatings to metals such as pipes, food box interiors, floor adhesives, as well as tooth fillers. After polymerization in 1957, BPA was found to form a very durable, strong and clear plastic, and it has been used in automobiles, food storage containers, water containers, electronics and safety equipment (Susana Almeida, 2018). Exposure to BPA occurs mainly through ingestion, inhalation, and dermal routes.

Unfortunately, plastics containing BPA can deteriorate due to incomplete polymerization, and at very high temperatures can cause BPA to dissolve from both parent materials. Thus, humans obtain BPA from various sources, such as plastic plates, plastic containers, food packed in plastic, tea/ coffee served in plastic cups or through contact with other BPA-containing products. When served hot, the BPA leaches and enters the human system. The effects of BPA mainly occur as a result of consuming contaminated foods and beverages that come in contact with epoxy resins or polycarbonate plastics. When bioactive BPA release from polycarbonate containers used for drinking water and other beverages is checked for permeability to water stored in new or used high quality polycarbonate containers, BPA from 0.20 to 0.79 ng per hour in polycarbonate water containers were found to be migrating at the same rates (Kubwabo, et al., 2009). Exposure to boiling water (100 ° C) increased the transfer rate of BPA by 55 times (Susana Almeida A.´.-G., 2018), and again the results show that the displacement of BPA at room temperature does not depend on whether the bottle has been used before or not (Hoa H. Le, 2008). This substance causes a variety of side effects such as cancer, endocrine, reproductive, metabolic and cardiovascular diseases. In addition, the formation of reactive oxygen species (ROS) due to BPA significantly impairs mitochondrial function. (Ulas Acaroz, 2019). BPA, as a toxicant, has a direct effect on the mechanism of resistance to oxidative stress in the cell. Because of its hormone-like properties, it can bind to specific receptors in target cells (Meli, 2020). Therefore, the tissue-specific effect of BPA is mainly due to its ability to disrupt endocrine and the expression of these specific receptors in target cells (Pang, 2019). BPA induced oxidative stress has been controlled by some types of enzymes which are important to prevent age related diseases. The sirtuin family of seven enzymes has also been linked to several antioxidant and oxidative stress-related processes and functions, including longevity, mitochondrial function, DNA damage repair, and metabolism. In addition, the fact that their deacetylase activity is dependent on NAD^+ (Chandra K. Singh, 2018) The highly conserved structure of sirtuins, as well as their NAD^+ dependence, suggests that these enzymes are a family of nutrient-sensing regulators cooperating in semi-redundancy to direct cellular metabolism in response to altered nutrition or oxidative stress (Athanassios Vassilopoulos, 2011).

They have different specific substrates including histones, transcriptional regulators and enzymes. Sirtuins can affect ROS production and increase resistance to its damaging effects. Oxidative stress has been shown to decrease SIRT1 expression in the cell (P.Sachdevae, 2013). Sirtuin family genes have also been found to be directly activated by polyphenols including quercetin, catechins, resveratrol and kaempferol. Investigation of polyphenolic sirtuin-activating compounds are thought to hold promise for reducing oxidative stress. SIRT2 is upregulated in response to calorie restriction and oxidative stress, SIRT3 reduces oxidative stress by increasing superoxide dismutase activity. Other two mitochondrial sirtuins, SIRT4 and SIRT5. SIRT6 is involved in DNA repair following oxidative stress through activation of the DNA repair enzyme. Polyphenols have been found to activate SIRT1, SIRT2, SIRT3, SIRT4 and SIRT7 protein and/or gene expression in cells and animal (Hiroyasu Yamamoto, 2007).

Among sirtuins, SIRT1 is the most widely studied and has been rated as of high importance for the prevention of degenerative diseases. Human SIRT1 is the largest protein of the sirutins family. Its molecular weight is 81.7 kDa and it consists of 747 amino acid residues (Joanna GERSZON, 2014). More than a dozen substrates have been described for SIRT1, including transcription factors and other regulators of age-related-degenerative diseases (Salvatore Fusco, 2012 Nov 15). SIRT1 also plays a major role in energy homeostasis in key metabolic tissues, which is potentially a manifestation of its ability to link metabolic status to transcriptional outputs. Hepatic metabolic derangements are key components in the development of degenerative diseases. Sirt1 is an important regulator of energy homeostasis in response to nutrient availability. It plays a vital role in the regulation of hepatic lipid homeostasis and that pharmacological activation of Sirt1 could be important for the prevention of obesity associated metabolic degenerative diseases (Akiko Satoh, 2011). Some researches also show that manipulation of Sirt1 levels in the liver affects the expression of a number of genes involved in glucose and lipid metabolism. Additionally, it has been demonstrated that modest overexpression of Sirt1 resulted in a protective effect against high fat induced hepatic steatosis and glucose intolerance (author1, 2011). In line with this, SIRT1 protein levels are increased in response to CR in many key metabolic tissues (Li*, 2013 Jan).

Many evidence derived from in vivo studies suggests that SIRT1 may mediate the effects of CR in rats. Rats mildly overexpressing SIRT1 seem to be protected from the development of metabolic disease when challenged with high-fat diets. Similarly, wild-type mice fed polyphenolic diet displayed prolonged lifespan and were protected against the development of metabolic disease. Treatment of Wistar rats with SRT1 720, a newly developed SIRT1 agonist, also mimicked the metabolic adaptations triggered by CR and conferred protection against metabolic disease (Auwerx, 2009)

The goal of the present study was to assess the antioxidant capacity and effect on SIRT1 protein expression of buckwheat-enhanced phenolic compounds in diet of Wistar male rats. Buckwheat (*Fagopyrum tataricum),* is a domesticated food plant raised in Asia, originated from the Yunnam province in southwest China and is widely consumed as flour or tea in many countries. Buckwheat is grown under almost limited environmental conditions during growth and developmental stages, especially at the early stage of development and flowering (Y Fujii, 2015). It is a highly nutritious whole that good source of superior quality protein, fiber, and energy. Among its health benefits, buckwheat may improve heart health, promote weight loss, and help manage degenerative diseases (Y.Z. Cai, 2004). Buckwheat contains many bioactive components and is rich in flavonoids, including orientin, vitexun, quercetin, and rutin. Especially, tartary buckwheat (*Fagopyrum tataricum*) is known to have about 80-folds higher rutin than common buckwheat (*Fagopyrum esculentum).* It had been widely studied that buckwheat promotes anti-oxidation, hypocholesterolemia, and anti-inflammation. Buckwheat (*Fagopyrum esculentum Moench, F. tataricum Gaertner*) groats and flour have been established globally as nutritional foods because of their high levels of proteins, polyphenols and minerals (YuTang, 2012). The bioavailability of nutritional compounds is based on their

gastrointestinal stability and the efficacy of their trans-epithelial transition (LeiChen1HuiCao2JianboXiao12, 2018). Polyphenols constitute one of the most numerous and ubiquitously distributed group of plant secondary metabolites and are generally involved in defense against stress, such as oxidative stress.

Buckwheat that is commonly consumed around the world has been continued to research upon for its the dynamic capacity to protect against age-associated disorders through a variety of important mechanisms. Numerous lines of evidence suggest that dietary polyphenols have the capacity to mitigate oxidative cellular damage due to their antioxidant capacity. The last includes the inhibition of the modulation of several cell survival/cell-cycle genes, and activation of deacetylase enzymes such as sirtuin family genes (F. Sarubbo, 2018). We were interested to study if buckwheat acts as a sirtuin regulator and works as portent functional food in rats. Functional foods are foods or food components which beyond providing basic nutritional needs, also have physiological/pharmacological benefits or reduce the risk of chronic diseases and also BPA induce stress. The present work was conducted to study effect of dietary buckwheat on SIRT1 protein expression and antioxidants in male Wistar rats. Buckwheat has significant amount of dietary fiber that is primarily indigestible. We hypothized that the indigestibility of buckwheat may mimic calorie restriction like effect and impact on SIRT1 protein expression. Further we wanted to understand if dietary buckwheat-led improvement in SIRT1 can offer any protection against BPA-induced toxicity in experimental animals. The aim of the present study was

- To study the effect of BPA on experimental animals
- To study the effect of dietary buckwheat on SIRT1 protein expression
- To study the effect of SIRT1 protein expression against detrimental effects of BPA

CHAPTER II

LITERATURE REVIEW

2.1 BPA Induced Oxidative Stress

Since the last decade, oxidative stress has become the most important research topic of many scientists working in the field of biology due to its role in the onset of various diseases. Oxidative stress, oxygen, and oxygen-supplied free radicals override the cell's natural antioxidant defenses, leading to damage to cell components, inactivation of metabolic enzymes, and disruption of signal transmission pathways (Esra Birben, 2012). Oxidative injury is well known to be associated both with the process of organism aging and with several chronic diseases that include among others, diabetes, atherosclerosis, age-related macular degeneration, cataract and Alzeheimer's disease (Kumar, 2015). Protein crosslinking, lipid peroxidation, mitochondrial dysfunction and induction of cell death pathways are among the proposed mechanisms of cellular damage due to this misbalance. Moreover, can be induced by BPA and environmental factors, leads to the accumulation of reactive oxygen/nitrogen species (ROS/RNS), further causing misbalance in pro-oxidant/antioxidant steady state or a significant decrease in the effectiveness of antioxidant protections (Anu Rahal, 2014). Oxidative stress is the imbalance between free radicals and antioxidants in the body, while free radicals are oxygen molecules that have an uneven amount of electrons. An uneven number allows them to react easily with other molecules, and because free radicals react easily with other molecules, they can cause large-chain chemical reactions in the body. These reactions are called oxidation and they can be beneficial or harmful (Legg, 2017) It is also the result of an imbalance between antioxidant defenses and reactive oxygen species (ROS), such as free radicals. Regardless of the species, ROS can damage biological macromolecules due to their high reactivity with -C-H, and other bonds. This can result in carcinogenesis, as well as lipid peroxidation, which in turn can lead to necrotic cell death (Małgorzata Nita, 2016). Eukaryotic cell has a variety of natural antioxidant defenses, such as reduced glutathione, ascorbic acid, thioredoxins, and α-tocopherol, and enzymes, like superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), play critical roles in redox reactions in the cell (O.M.Ighodaroa, 2018). Oxidative stress induces modifications of cellular proteins, thereby altering their functions and causes a state of susceptibility to a wide range of disorders. It causes damage to biomolecules including DNA, lipids and proteins and contributes to the pathology of many diseases including neuronal degeneration, autoimmune diseases, cardiovascular dysfunction, accelerated aging, the progression of cancer and conditions of the reproductive system including both male and female infertility (Durairajanayagam, 2017). BPA is well established as an endocrine disruptor due to its role as an estrogen mimic. Endocrine disruptors can act through nuclear receptors, nonnuclear steroid receptors such as membrane estrogen receptors, non-steroid receptors such as neurotransmitter receptors, orphan receptors, and many other pathways that converge on endocrine and reproductive systems. Even at low concentrations, BPA demonstrates a potency similar to estradiol in endocrine-receptor-dependent signaling pathways, due to the phenol in its structure (Paul S. Cooke, 2013). When BPA is ingested orally, passes through the gastrointestinal tract and liver before entering the internal tissues. However, detoxifying enzymes have been proven in several experiments to play a crucial role in the destruction of intestinal wall and liver-derived chemicals (Muna S. Nahar, 2012). The cell's ability to maintain balance with the reduction and oxidation (redox) of chemical compounds plays an important role in all aspects of cell development, and growth. Normal cellular metabolism induces ROS, such as superoxide anions, peroxides, and hydroxyl radicals, and cells have created highly tuned pathways to use small amounts of these species to regulate genes and shed

excess ROS to prevent harmful effects (Kurutas, 2016). This important oxygen balance is maintained by many components in the cell and is highly regulated and coordinated. However, when this balance is disturbed by BPA or environmental toxins, the same ROS that was once beneficial to the cell can now cause insensitivity to mutations, cell growth and cell death signals. BPA has been shown to lower antioxidant defenses, (Gokul Prasanth, 2012). Taken together, these results suggest that BPA may have an important effect on oxidant-antioxidant scale tipping, whose net effect is an increase in oxidative stress. In addition, increased levels of oxidative stress are associated with aging, cardiovascular disease, neuronal degeneration, and the development and progression of cancer (Gassman1). According to some reports, perinatal BPA exposure leads to reduced levels of gut bacterial diversity and bacterial metabolites and elevated gut permeability—three common early biomarkers of inflammation-promoted chronic diseases. Furthermore intensified inflammation plays an important role in diseases caused by BPA (Lavanya Reddivari, 2017).

2.2 Generation of Reactive Oxygen Species

Reactive oxygen species are chemically reactive chemical species containing oxygen. Examples include peroxides, superoxide, hydroxyl radical, singlet oxygen, and alpha-oxygen. In a biological context, ROS are formed as a natural byproduct of the normal metabolism of oxygen and have important roles in cell signaling and homeostasis (Yosef Dror, 2020). However, during times of environmental stress (UV or heat exposure) or BPA induce stress, ROS levels can increase dramatically. This may result in significant damage to cell structures. Cumulatively, this is known as oxidative stress. Effects of ROS on cell metabolism are well documented in a variety of species. These include not only roles in apoptosis (programmed cell death) but also positive effects such as the induction of host defense genes and mobilization of ion transport systems. This implicates them in control of cellular function. In particular, platelets involved in wound repair and blood homeostasis release ROS to recruit additional platelets to sites of injury. These also provide a link to the adaptive immune system via the recruitment of leukocytes (P, 2019). Reactive oxygen species are implicated in cellular activity to a variety of inflammatory responses including cardiovascular disease. They may also be involved in hearing impairment via cochlear damage induced by elevated sound levels, in ototoxicity of drugs such as cisplatin, and in congenital deafness in both animals and humans (Mukherjea, 2011). ROS are also implicated in mediation of apoptosis or programmed cell death and ischemic injury. Specific examples include stroke and heart attack. In general, harmful effects of reactive oxygen species on the cell are most often: damage of DNA or RNA, oxidations of polyunsaturated fatty acids in lipids (lipid peroxidation), oxidations of amino acids in proteins, oxidative deactivation of specific enzymes by oxidation of co-factors (Małgorzata Nita, 2016). In aerobic organisms the energy needed to fuel biological functions is produced in the mitochondria via the electron transport chain. In addition to energy, reactive oxygen species (ROS) with the potential to cause cellular damage are produced (Nabi, 2014). ROS can play key role with damaging lipid, DNA, RNA, and proteins, which, contributes to the physiology of aging. They are produced as a normal product of cellular metabolism. In particular, one major contributor to oxidative damage is hydrogen peroxide (H_2O_2), which is converted from superoxide that leaks from the mitochondria (Zuhl., 2015). Catalase and superoxide dismutase ameliorate the damaging effects of hydrogen peroxide and superoxide, respectively, by converting these compounds into oxygen and hydrogen peroxide (which is later converted to water), resulting in the production of benign molecules (Byung-Mu Lee, 2017). However, this conversion is not 100% efficient, and residual peroxides persist in the cell. While ROS are produced as a product of normal cellular functioning, excessive amounts can cause deleterious effects.

2.3 Oxidative injury of cells

Aerobic organisms have integrated antioxidant systems, which include enzymatic and nonenzymatic antioxidants that are usually effective in blocking harmful effects of ROS. During conditions of metabolic stress, like obesity and metabolic syndrome, an oxidative stress environment is created, mainly due to a state of chronic inflammation. Oxidative stress can affect the activity of sirtuins at different levels: expression, posttranslational modifications, protein-protein interactions, and NAD levels (Leonardo Santos 1. C., 2016). Mild oxidative stress induces the expression of sirtuins as a compensatory mechanism, while harsh or prolonged oxidant conditions result in dysfunctional modified sirtuins more prone to degradation by the proteasome. SIRT1 can be potential link between cellular metabolic status and adaptive transcriptional responses. It is already established that SIRT1 proteins exert their effects through two different pathways namely histone modifications and nonhistone substrates (author P. M.-R., 2013). Sirtuins constitute a family of highly conserved nicotinamide adenine dinucleotide (NAD+)-dependent enzymes that deacetylate histones and residues of acetylated lysine. A common feature of the activity of sirtuins is their dependence on intracellular ratio of NAD+ and its reduced form NADH (Kristin A Anderson, 2017). Sirtuins also have been shown to play an important role in the defense mechanisms against pathological conditions, but the excessive generation of free oxygen radicals may damage tissues and also damage proteins, leading to the structural alteration and functional inactivation of many enzymes and receptor proteins involved in cell signaling (Aprioku*, 2013). From some recent studies, it is now clear that this generation of ROS by BPA exposure depends on cell types and which hormone receptor is being found in that particular cell type. Environmentally persist low level of BPA exposure might be able to cause oxidative damage by disturbing the balance between reactive oxygen species and antioxidant defense system, resulting in the development of oxidative stress-related diseases (Meli, 2020). Experimental data have shown BPA can induce the generation of ROS through the enzymatic and non-enzymatic formation of radicals. BPA induce mitochondrial dysfunction in the liver is thought to be caused by an increase in oxidative stress and inflammation. Cell death, DNA mutation, replication errors, and genomic instability can occur if the oxidative DNA damage is not repaired before DNA replication (Dinari Tiwari, 2017). BPA-induced oxidative stress affects SIRT1 expression, which then affects the expression and activity of downstream proteins, resulting in oxidative damage. Upregulation of SIRT1 expression by SIRT1 activator can generally alleviate the toxicity of BPA (Jing Xu, 2018). SIRT1 can interact with proteins in various signal transduction pathways and regulate biological, physiological, and pathological processes. For example, SIRT1 can reduce the release of inflammatory factors by inhibiting the expression and activity of NF-κB in the NF-κB signal transduction pathway, thus alleviating the inflammatory damage caused by some toxicants. Therefore, an in-depth study of the role and mechanism of action of SIRT1 in toxic damage caused by poisons may provide new insight into therapeutic strategies to limit the toxic damage caused by poisons. SIRT1 can catalyze the deacetylation of acetyl lysine of histone substrate and some non-histone substrates to regulate gene expression. It can participate in the regulation of apoptosis, the inflammatory response, oxidative stress, energy metabolism, and other processes by regulating different pathways, playing an important role in toxicological damage.

2.4 SIRT1-mediated signaling pathways

Based on the key role of sirtuins in the regulation of metabolic responses, we can view changing in the redox status of the cells affect the activity of sirtuins and what are the biological consequences of these alterations. While the nonhistone modification exhibits that once SIRT1 is activated it mediates intracellular responses that promote cell survival, enhance the repair of damaged DNA, and reduce cell division (Jiho Jang, 2017). Also, SIRT1 is associated with the heterochromatin regions where they promote deacetylation of histone. It can be inferred that owing to multiple molecular functions performed by SIRT1namely deacetylation, epigenetic modifications, and transcription factor modulation (Farnham, 2011) (Vittorio Sartorelli1, 2018). In histone modification, many ageing related effects are caused by chromatin changes. Because SIRT1 is localized mainly in the nucleus, its physiological actions are partly mediated by its ability to deacetylate nucleosomal histones at specific residues (Lin*, 2015). As SIRT1 lacks a DNA-binding domain, it is subjected to target promoters by sequence-specific transcription factors so as to incorporate chromatin remodeling and subsequently regulation of gene expression (Farnham, 2011). Experiments indicated that for every acetyl lysine group that is removed, one molecule of NADC is cleaved, nicotinamide and O-acetyl-ADP-ribose are produced (Jonathan M. Solomon, 2015). Therefore, SIRT1 appears to possess two enzymatic activities; first the deacetylation of a target protein and second the metabolism of NADC. This distribution helps us to understand the underlying mechanism attributed to sirtuin and its effect on health. In all living organisms, cellular energy is produced and expended using universal “energy currencies” such as ATP and NADH (Falkevall, et al., 2017). The tight balance between such anabolic and catabolic pathways ensures that cells do not deplete essential energy stores, which would ultimately cause cellular damage or death. Sirtuins are significantly involved in mammalian energy homeostasis. The activity of SIRT1 is highly regulated towards the environmental factors that may influence it (Lee, 2019). A feeding regime that is reported to accelerate the sirtuin activation is caloric restriction, which corresponds to a lowering of 20–40% calories below ad libitum intake without inducing malnutrition (Shubhra Pande1, 2017). The levels of sirtuin have been reported to enhance in fasting mice. Much difference in opinion has been reported in context to understand how calorie restriction can affect the life span. PGC-1α is a transcription factor co-activator that affects most cell metabolic pathways. It influences mitochondria respiration, the reactive oxygen species defense system, and fatty acid metabolism by interacting with specific transcription factors. Studies have shown that SIRT1 can enhance tissue antioxidant capacity by activating the transcription of PGC-1α and inducing the expression of superoxide dismutase (SOD) and glutathione peroxidase (GSH-PX) in cells (Patel SA1, 2014). Therefore, when toxic substances directly act on SIRT1 to reduce its expression, it can reduce the antioxidant capacity of tissues and cause oxidative damage to the body. Some other researchers found that early lead exposure could reduce phosphorylated PGC-1α in the mouse cerebral cortex and SIRTl expression in the nucleus of cerebral cortex cells, increase the retention of PGC-1α in the cytoplasm, reduce the activity of GSH-PX and the GSH content, and reduce the antioxidant capacity (Zhihua Ren, 2019). Excessive fluoride can also inhibit SIRT1, significantly downregulate the protein expression level of SIRT1, and cause central nervous system oxidative damage through the SIRT1/PGC-1α pathway. SIRT1 can also regulate the function of PGC-1α in cells by regulating the acetylation and activity level of PGC-1α, as well as regulation of downstream transcription factors such as nuclear receptor peroxisome proliferator-activated receptor (PPAR), estrogen-related receptor (ERR), nuclear respiratory factor (NRFs), and mitochondrial transcription factor A (Tfam), further affecting mitochondrial production and function, and regulating the metabolism of glucose and lipids. In addition, structural damage or dysfunction of mitochondria also leads to the initiation of apoptosis, so SIRT1 can regulate the functional state of mitochondria and indirectly control apoptosis by regulating the acetylation level of PGC-1α. Regarding BPA

toxicity damage, studies have found that BPA treatment can obviously inhibit the expression of SIRT1 (Rosanna Chianese, 2018) and increase PGC-1α acetylation levels, damaging mitochondria and leading to mitochondrial dysfunction, and eventually inducing cell death processes such as apoptosis and necrosis, which may also be an important cause of hepatotoxicity induced by BPA. Therefore, in the oxidative damage caused by some poisons, the SIRT1-mediated PGC-1α pathway can play an important role by regulating the body's antioxidant capacity and mitochondrial production and functional status. NF-κB is the master switch of the inflammatory response, which is usually connected to inhibitory protein inhibitor of NF-κB (IκB) in the form of a p65/p50 dimer (Ting Liu, 2007). When stimulated, p65/p50 can be activated and transferred to the nucleus to regulate the transcription of various downstream inflammatory factors. The p65 subunit of NF-κB is the direct target of SIRT1, which, through deacetylation, can control the acetylation level of NF-κB p65 to regulate the transcription level of the downstream genes, including those encoding IL-1, tumor necrosis factor α (TNF-α), IL-8, IL-6, and other inflammatory factors, thus regulating the inflammatory response (Andrea Oeckinghaus1, 2009). In addition, NF-κB is also involved in the regulation of apoptosis, and SIRT1 regulates anti-apoptosis-related gene expression through NF-κB. Excessive amount of BPA can reduce the expression of SIRT1, so that NF-κB cannot be deacetylated, resulting in activation of the NF-κB signal, which causes neuronal apoptosis and central nervous system damage (Mark P. Mattson1, 2001). It can be seen from the above that SIRT1 can regulate apoptosis by controlling the level of deacetylation of NF-κB, thus affecting the toxic damage of some toxicants. However, the SIRT1/NF-κB pathway mainly participates in the toxic damage process of toxicants by the inflammatory response. The FOXO protein family is widely involved in cell signal transduction, growth and development, apoptosis, and antioxidant stress, among which FoxO1 and FoxO3 are the most common. This family of proteins can activate or inhibit a variety of target genes, such as p27kip1 and cyclin D (CCND) CYR61, which regulate the cell cycle, the *bim* and *fasL* genes that mediate apoptosis (S. Mazumder, 2004). The complex interaction between SIRT1 and FOXO protects against oxidative stress (Yu-Qiang Wang 1, 2015). On the one hand, SIRT1 upregulates the deacetylation of FOXO, enhances FOXO-induced cell cycle arrest, activates and promotes the FOXO/MnSOD pathway, increases the expression of manganese superoxide dismutase (MnSOD) and catalase (CAT) to resist oxidative stress, and promotes the repair of DNA damage during replication (Candida Fasano, 2019). On the another hand, after deacetylation of FOXO by SIRT1, FOXO can be degraded by ubiquitination, reducing the level of FOXO and inhibiting the ability of FOXO to induce cell death, thereby ultimately protecting cells from oxidative stress damage (Hiroak iDaitoku, 2011). In BPA-induced toxic effects on SIRT1 cause a decrease in expression, and a decrease in the level of FOXO deacetylation leads to an increase in apoptosis, leading to damage. After activation of the SIRT1/FOXO pathway, the level of FOXO deacetylation not only regulates the oxidative stress of the body, but also involves the control of cell apoptosis and the cell cycle, which is a complex and interactive process (author P. S., 2011). Therefore, the study on the role of this pathway in the toxic injury of related toxins should be more comprehensive and systematic. Nuclear factor E2-related factor 2 (Nrf2) is widely regarded as a transcription factor activated by oxidative stress that induces the coding of a series of antioxidant protective proteins and promotes the regulation of redox conditions in cells. In addition, Nrf2 is also an important negative regulator of inflammatory cytokine activation and interleukin-1-mediated vascular inflammation, and therefore participates in the process of inflammation (JunWangbXiuwenTang, 2017). Some studies have shown that Nrf2 can be regulated by acetylation, while SIRTI can activate Nrf2 transcriptional activity and upregulate Nrf2 downstream gene expression of genes such as those encoding SOD and GSH (Zhihua Ren #. H., 2019). Conversely, downregulation of SIRT1 expression significantly reduced Nrf2 protein expression. Regarding the oxidative stress caused by BPA, some studies have found that overexpression of SIRT1 can deacetylate NRF2, increase the stability of Nrf2, promote the transport of Nrf2 to the

nucleus, promote the transcriptional activity of Nrf2, enhance the resistance of cells to oxidative damage, and play a protective role in the AEC-II injury of mice caused by BPA poisoning (Syed Zahid Ali Shah, 2018). It can be seen that the SIRT1/Nrf2 pathway can antagonize the oxidative damage caused by some toxicants by enhancing the antioxidant capacity of the body. p53 can regulate the expression of a large number of downstream target genes, which in turn affects cell cycle organization, apoptosis, differentiation, and a number of other processes. SIRT1 enhances the expression of MnSOD by deacetylating p53, thereby increasing cellular antioxidant capacity (Zhihua Ren H. H., 2019). It is also negatively regulated by p53. When cells are under oxidative stress, SIRT1 can deacetylate the lysine residue at position 382 of the p53 protein and inhibit the activity of p53, thereby inhibiting the transcription of downstream target genes dependent on p53, such as CDKNIA and BAX, reducing cell apoptosis. In the oxidative stress caused by BPA, SIRT1 can regulate the deacetylation level of p53, which can affect the antioxidant capacity of cells and regulate cell apoptosis (Atsushi Kuno, 2013). Studies have shown that SIRT1 plays an essential role in protection against BPA-induced oxidative stress and mitochondria-dependent apoptosis in cells (Rosaria Meli, 2020).

2.5 Sirtuin expression enhance by Calorie restriction

The beneficial health outcomes of CR resemble those that are induced by antioxidants in a number of animal models, suggesting that the molecular pathways by which antioxidants act are similar to those activated by CR (Kerry Bone, 2013). Recently, it was suggested that the sirtuin genes could be the common mediators that explain both the effects of antioxidants and CR pathways. Which CR condition, mild or severe, is more relevant to the one applied to mammals (30–40% reduction in calorie intake), one possibility is that when mammals are calorie restricted, tissues with different energy demands experience varying degrees of CR (Jasper Most, 2017). When food is scarce, mammals may have to redistribute the limited resource to maintain survival and shut down unnecessary energy expenditures, such as growth, synthesis and reproduction. Thus, tissues necessary for basic survival, such as the muscle, the heart, and certain brain regions are protected from starvation and experience mild CR. However, tissues for synthesis, such as the liver and the pancreas, are likely to experience severe CR. Although in the circulation, levels of glucose are roughly the same throughout the body, tissues may experience varied degrees of CR due to different capacities for glucose uptake (Gerich, 2010), additionally, emerging genetic evidence suggests that mammalian sirtuins are required for the CR response. SIRT1 may be required for increased physical activity of CR rats, as SIRT1 knockout rats do not have increased activity (Ruben Nogueiras, 2012). It has been thought that CR extends lifespan by decreasing metabolism and the associated production of damaging reactive oxygen species. However, this traditional view has been challenged by recent findings indicating that CR in fact increases metabolism. Evidence for changes in oxygen consumption in CR rats is controversial, as the rats experience drastic alterations in body weight and composition (S W Corbett, 1986). When energy is abundant, instead of storing excess energy in the form of ethanol, animals store it as fat. During CR, animals turn on fatty acid oxidation and switch fuel usage from glucose to fatty acids. Since fatty acids are more reduced than glucose, fatty acid oxidation will consume more oxygen per carbon than glycolysis. At the cellular level, increased mitochondrial biogenesis in CR tissues suggests a tissue-specific increase in metabolic rate of CR animals (Rai Ajit K. Srivastava, 2012). Antioxidants treated rats have increased numbers of mitochondria, decreased levels of blood glucose and insulin, increased glucose tolerance and insulin sensitivity, and improved motor activity, reminiscent of CR effects. In addition to its interaction with SIRT1, antioxidants are known to interact with many other proteins and pathways involved in energy balance, such as mitochondrial ATP synthase, complex III, fatty acid synthase, and AMP kinase (Rai Ajit K.

Srivastava, 2012). An important question is whether the CR-mimicking effects of polyphenols are mediated by SIRT1 *in vivo*. Newly identified SIRT1 activators structurally unrelated to antioxidants, such as SIR1720, have similar effects in obese rodents, such as improved insulin sensitivity and mitochondrial capacity.

2.6 Cell enzymatic defense system against oxidative stress

Sirtuin genes are reported to act as sensors that detect cellular energy availability leading to metabolic benefits, as calorie restriction extends lifespan in organisms ranging from yeast to mammals. It is shown that the mammalian Sir2 orthologue, SIRT1 (sirtuin 1), activates a critical component of calorie restriction in mammals; that is, fat mobilization in white adipocytes. Recent studies suggest a key role for the mammalian SIRT1 in adequate cellular response to metabolic stress such as nutrient deprivation or overload and that SIRT1 and its activators play role in protection from the detrimental effects of metabolic stressors (Nilika Chaudhary, 2012). A key role in the regulation of adipogenesis is played by the nuclear receptor PPAR-γ (peroxisome proliferator-activated receptor-gamma). Indeed, upon food withdrawal SIRT1 protein binds to and represses genes controlled by the fat regulator PPAR-γ, including genes mediating fat storage. SIRT1 represses PPAR-γ by docking with its cofactors NCoR (nuclear receptor co-repressor) and SMRT (silencing mediator of retinoid and thyroid hormone receptors). Mobilization of fatty acids from white adipocytes upon fasting is compromised in SIRT1 (+/-) mice. In differentiated fat cells, upregulation of SIRT1 triggers lipolysis and loss of fat (Picard F, 2004). As reduction in fat is sufficient to extend murine lifespan, the above-mentioned results provide a possible molecular pathway connecting calorie restriction to life extension in mammals. In a study using liver-specific SIRT1-knockout mice, some challenge the assumption that calorie restriction always activates SIRT1 in all tissue types by demonstrating that SIRT1 activity is reduced in the liver during calorie restriction, yet activated when mice are fed a high calorie diet (Robert Fried, 2018). The study determines that liver-specific SIRT1-knockout mice have at least some protection, compared with wild-type mice, from accumulating fat while on a high-calorie diet. In contrast, while under calorie restriction, the liver-specific knockout rats have the same phenotype. These observations suggest that hepatic SIRT1 may be inactivated during calorie restriction in normal mice and activated while on a high-calorie diet, opposite to what occurs in the muscle and the white adipose tissue that can be explained by different redox status and NAD/NADH ratio in the liver from other tissues under study conditions. It might raise the interesting possibility that SIRT1 inhibitors specifically targeted to the liver may be of benefit in treating obesity (Danica Chen, 2008). The concept that activation of SIRT1 can result in loss of body fat without affecting the calorie intake could open the door for novel treatment for obesity and related diseases. Further, sirtuin may be believed as a promising biomarker to reveal the nutritional status in terms of fat accumulation and oxidation and its modulation will beneficially impact the storage and metabolism of fat in the body. The effect of sirtuin on overall metabolism is multidimensional and its metabolic functions reveal that their effect on degenerative diseases is extremely crucial. Sirtuins influence the immune system also by reducing inflammation in multiple tissues particularly macrophage, whereas the reduction of SIRT1 in hepatic cells caused in increased local inflammation. Some scientific articles revealed that a mice fed a high-fat diet when administered with SIRT1, resulted in improved liver functions and metabolism.

In the brain, SIRT1 functions as a potential link between the pituitary hormones and calorie restriction longevity pathways in mammals. Many changes induced by SIRT1activation are related to increased mitochondrial metabolism and antioxidant protection in the fasting fish (Baur, 2010). It is worth noting that SIRT1 overexpression downregulated the pro-inflammatory genes in mice, whereas obesity with chronic inflammation was associated with reduced levels of SIRT1. This finding advocates and high-lights the capability of sirtuin as a biomarker for lipid

accumulation led inflammations in biological systems. The members of sirtuin families being NADC-dependent deacetylases, participate in many cellular processes as cell proliferation, senescence, and stress response (Shin-Hae Lee, 2019). They might play either a promoting or suppressing role, depending on the organ or even the species. Expression of SIRT1 increases in prostate cancer and acute my elocytic leukemia (DENG-FENG YU, 2016). An enhanced overexpression of SIRT1in colonic tubular adenoma was observed and it was advocated as a useful biomarker in the diagnosis of high-grade dysplasia and invasive carcinoma. Other group of researchers observed that the activity of SIRT1 and SIRT2 protein was significantly increased in the cancer cell lines of lungs as compared with non tumor epithelial cells of the lungs (Ivana Grbesa, 2015). The expression of SIRT1and SIRT2 proteins was also found to increase in tumor cells of lungs than normal lung cells. These findings even suggested that SIRT1 inhibitors may act as potential anticancer agents and that the potential tumor suppressive effects of SIRT1 need to be kept in mind while considering SIRT1 inhibitors for cancer treatment. There are multiple studies that demonstrated that the possible regulatory mechanism of SIRT1 on the cancer gene is associated with tumor protein p53. As weal ready know that the p53 protein is a tumor suppressor protein (Mohammad Athar, 2011). Its lowered expression or mutation leads to enhanced risk of cancer. Deacetylation of p53 by SIRT1 is reported to play an important role in preventing p53 activation and thus promoting cancer (Fang, 2013). This is how SIRT1 impacts the activity of p53 gene and gets highlighted as a cancer promoting agent. But there is a considerable paradox in this regard. Several contradicting studies have indicated that the p53 inactivation by SIRT1 actually promotes cell survival during stress and that SIRT1 arrests p53 induced apoptosis by p53 deacetylation and induction of manganese superoxide dismutase. Despite clear inhibitory effect of increased SIRT1 expression on tumor suppressors like p53, other studies have suggested that SIRT1 may have tumor suppressive functions as well (Jingjie Yi1, 2010). This can be partly explained by studies conducted by where they observed that SIRT1 offers protection against oxidative stress through modulation of fork head transcription factors in some cells. Although researchers observed that SIRT1 protects cells against oxidative stress by increasing the activity of antioxidant enzyme catalase (Antero Salminen, 2013). The calorie restriction helps to combat oxidative stress through SIRT3-mediatedenhancement of super oxide dismutase (SOD) activity. Also, SIRT1 overexpression increases the tolerance against free radical toxicity in neuronal cells. Some studies about polyphenols which was reported to improve chances of cell survival by stimulating SIRT1-dependent deacetylation of p53 (Konrad T Howitz 1, 2003).The expression and activation of SIRT1 can be influenced by several cellular conditions such as calorie restriction, exercise, and oxidative stress in the cell. SIRT1 uses NADC as a substrate, but the level of NADC can also control the deacetylating activity of SIRT1. Moreover, the activity of SIRT1 may depend on the cell process and cell-type studied. So can it be justified to propose that sirtuin levels increases during cancer as a part of body's homeostasis and performs protective mechanism to fight against cancer and induce longevity as sirtuins. This aspect of research is scattered with contradictions and bidirectional views, therefore it needs more focus and insight to actualize the role of sirtuin in degenerative diseases. SIRT4 has been observed to modulate the metabolism of Non-esterified ("free" or unsaturated) fatty acids (NEFA) (Frank K. Huynh, 2018). The adipose tissues release NEFA, triggering oxidative stress that results in endothelial dysfunction, early atherosclerosis, culminating to risk factors of coronary artery disease. Lowering activity of SIRT4 has been associated with an increased free fatty acid oxidation in liver and in muscle (Yumei Han, 2019). This finding indicates that an enhanced level of SIRT4 may qualify as an indicator of better antioxidant status of the organism in terms of concentration of NEFA. Researches has reported that sirtuin reduce the reactive oxygen species (ROS) by modulating the acetylation of the respiratory chain, stimulating mitochondrial SOD and isocitric dehydrogenase which generates NADPH for glutathione pathway (A. Y. Andreyev1*, 2015). Such reports establish the significant antioxidant potential of sirtuins and they had shown that

all the seven sirtuins are found in detectable limits in all human tissues, moreover the impact of sirtuin on most of the tissues is traceable so we must ascertain the metabolomics performed by sirtuins and examine it in detail.

2.7 Effect of antioxidants on sirtuin defense functions

The utilization of proteomics in nutritional research includes comprises of dimensions that identifies the composition and characteristics of proteins ingested, that is further detailed by digestion and absorption of nutrients in the gastrointestinal tract. Advanced proteomics research investigates nutrient metabolism (synthesis and catabolism) and regulation, transport of nutrients, tissue-specific metabolism of nutrients, role of phytochemicals in growth, signal transduction, cellular defense against oxidative stress, cell proliferation, differentiation, apoptosis, and gene expression in response to nutrients and other dietary factors (which may impact absorption of nutrients in the body) (Junjun Wang, 2006). Noteworthy are the families of silent information regulators (SIRT) that encode for genes which promote body's defense during positive physiological stress like calorie restriction. As well, studies on hepatocytes have demonstrated quercetin's antioxidant potential where it increased antioxidant capacity of the hepatocytes, decreased pro-oxidant and inflammatory mediators and modulated expression of several antioxidant genes (Anu Rahal 1. A., 2014). Thus, given these encouraging findings of quercetin as a potent antioxidant, it is equally important to test this substance in its potential to ameliorate other diseases. Therefore researchers are hoping to find a way to concentrate the effect into a safe dose within an effective therapeutic range. Additionally, the protective role of polyphenols against a number of hepatic injuries (e.g. cholestasis) due to oxidative damage of primary rat hepatocytes was reported by several authors including our own in results. In addition, intraperitoneal administration of polyphenols in rats with ligated bile ducts maintained antioxidant defenses and reduced liver oxidative damage and ductular proliferation (Emanuelle Kerber Vieira, 2014). Also, other naturally occurring substances of plant origin have been claimed to possess hepatoprotective actions and these include curcumin, catechin, quercetin and rutin. Polyphenols have been found to interact with multiple molecular targets, many of them associated with inflammation and immunity, thus its potential use in therapy of immune-mediated diseases was also reported. Generally, Polyphenols have been identified as a phytoalexin, antioxidant, cyclooxygenase (COX) inhibitor, peroxisome proliferator-activated receptor-alpha (PPAR-α) activator, endothelial nitric oxide synthase (eNOS) inducer, silent mating type information regulation 2 homolog 1 (SIRT1) activator belonging to a superfamily known as sirtuins whose name stems after their homology to the Saccharomyces cerevisiae gene silent information regulation-2 (Sir2) (Yoshie Takizawa, 2013). This is related to developing strategies to protect against diet-induced metabolic imbalance. It was suggested that the hypothalamus is a target for developing novel drugs that suppress SIRT1 degradation, as a strategy for treating metabolic syndrome (Jose M Villalba, 2012). Deciphering the basic mechanism of sirtuin activators is essential to develop certain strategies to alter sirtuin activity. This is true regardless of the apparent controversy of whether in vitro activation of SIRT1 is direct or not, depending on the experimental design, and whether sirtuins may play a major role in longevity. The numerous studies on their positive effects against age-related diseases, obesity and other metabolic disorders are still valid, promising to positively influence the development of treatments to improve human health. In fact, polyphenols are attractive molecules that represent potential epigenetic targets in drug discovery with allosteric mechanism. Epigenetics, at the molecular level, involves the dynamic regulation of covalent modifications to the histone proteins and DNA that influence gene expression and silencing, apoptosis, maintenance of stem cell pluripotency, X-chromosome inactivation and genomic imprinting without affecting DNA sequence (Daan J. A. Crommelin, 2019). Therefore, epigenetics is considered as the conduit from genotype to phenotype. The epigenetic techniques emphasize the

histone code and examine the utility of small molecule modulators of enzymes that modify histones and DNA. The dynamic remodeling of chromatin is essential to most DNA-based nuclear processes and it comes as no surprise that epigenetic changes are implicated not only in normal development but also in various diseases (Diane E. Handy, 2011). The large set of structural knowledge already obtained on epigenetic targets pave the way for drug design studies to act on major biological processes such as development, aging, diseases and cancer. Among the basic knowledge gained on catalytic domains of the main histone modifying enzymes are histone deacetylases. Histone deacetylases (HDACs) catalyze the removal of acetyl groups from epsilon-N-acetylated lysine in a nucleosomal context, ensuring the reversibility of histone acetylation (Yoshida2, 2014). Histone deacetylation is often associated with transcriptional repression and gene silencing, since it promotes chromatin of higher order structures and the recruitment of silencers. Among this superfamily is a HDACs class III which includes NAD+ dependent deacetylases known as sirtuins (silent information regulator 2-related proteins). Polyphenols as an allosteric modulators of the regulatory target SIRT1. Allostery has been established as a fundamental mechanism of regulation in all organisms, governing a variety of processes that range from metabolic control to receptor function and from ligand transport to cell motility (Shaoyong Lu 1, 2019). For example, small molecule activators of SIRT1 have been developed as therapeutics for the treatment of type 2 diabetes (Philip D Lambert, 2007). Similarly to polyphenols, these compounds bind to the SIRT1 enzyme-peptide substrate complex at an allosteric site amino-terminal to the catalytic domain and increase the affinity for acetylated substrates.

Many articles exist that deal with the biology and pharmacology of resveratrol, including many recent reports dealing with the molecular mechanisms of polyphenols´s cytoprotection. Several potential beneficial effects of polyphenols could be attributed to its general effects as antioxidant, anti-inflammatory, alteration of drug metabolizing enzymes, inhibition of cyclooxygenases, and importantly specific effects on proteins and/or signaling cascades as SIRT1 and AMPK. In addition, recent reports indicate intricate relationships between resveratrol, nuclear factors, autacoids and cytoprotection in various cells, tissues or organs. For instance in one study, polyphenols suppressed lipopolysaccharide (LPS)-induced nuclear translocation and activation of nuclear factor kappa B (NF-κB) in C6 microglia demonstrating an inhibiting effect of polyphenols on pro-inflammatory responses in microglia (Milne JC, 2007). Similar finding about the protective effect of polyphenols as an inhibitor of NF-κB-mediated vascular cell adhesion molecule induction was reported. Recently, NF-κB was suggested as a target for drug therapy in liver diseases where polyphenols was among several agents that inhibits the aforesaid transcription factor (Muriel, 2009). The fact that NF-κB has been associated with the induction of pro-inflammatory gene-expression makes research on agents which inhibit NF-κB an interesting topic. Even other findings on experimental animals demonstrate that treatment with resveratrol can reduce structural airway remodeling changes and hyperreactivity which has important implications for the development of new therapeutic approaches to asthma. However, NF-κB has been considered as an anti-inflammatory factor in certain situations and thorough understanding of the function of the diverse NF-κB factors is needed to examine its relation with resveratrol or similar drugs with cytoprotective effects. In our studies, we have investigated effects of resveratrol pretreatment on the enhancing action of D-Galactosamine (D-GalN) on LPS-induced liver failure in rats and in immobilized perfused hepatocytes as a short term bioreactor model with a chemical prooxidant. Liver function was assessed together with plasma nitrite as a measure of NO, estimation of nonenzymatic and enzymatic antioxidants was performed in plasma and liver homogenate and morphological examinations were performed using light and electron microscopy. Observations related to pharmacological increases of inducible nitric oxide synthase (NOS-2) / NO and inducible heme oxygenase (HO-1) / carbon monoxide (CO) in fulminant hepatic failure and modulation by resveratrol were followed up by real-time reverse transcription PCR (RT-PCR) in liver tissue. In the last study we

found that reduction in NO production, down-regulation of NOS-2 expression, modification of oxidative stress parameters and modulation of HO-1 are among the mechanisms responsible for the cytoprotective effect of resveratrol in the LPS/D-GalN liver toxicity and tert-butylhyroperoxide-induced hepatocyte toxicity models. This led to the overall improvement in hepatotoxic markers and morphology after the hepatic insult by resveratrol pretreatment.

2.8 Buckwheat act as a sirtuin regulator functional food

Buckwheat (*Fagopyrum esculentum* Moench) is an alternative crop belonging to the *Polygonaceae* family, also a good option for weight management. In comparison to antioxidant activity of frequently used cereals, buckwheat has been reported to possess higher antioxidant activity, mainly due to high rutin content. Rutin is a glycoside of the bioflavonoid quercetin with various protective effects due to its antioxidant and anti-inflammatory potential. Antioxidant compounds in plants, for example, tocopherols, carotenoids, and other phenolic compounds, are effective in the protection against oxidative damage toward membranes that contain polyunsaturated fatty acids. Therefore, many plants were investigated as sources of natural antioxidants; a great variety of compounds have been isolated, many of which are phenolic compounds. For the phenolic compounds in buckwheat, flavonols such as rutin, hyperin, quercitrin, and quercetin were isolated from its immature seeds, and flavones such as vitexin, isovitexin, orientin, and isoorientin were detected in its seedlings (Masahiro Koyama, 2013). On the other hand, some other researchers reported on the occurrence of syringic acid, p-hydroxybenzoic acid, vanillic acid, p-coumaric acid, and proanthocyanidins in the branaleurone layer of the buckwheat seed (Klepacka J, 2011). However, the study of antioxidant activity of phenolic compounds in this crop thus far has been superficial. Also depending on dietary fibres (they may be useful because they prolong the intestinal phase of nutrient digestion and absorption. Dietary fibre mainly occurs in cell walls, brans (whole cereals) or hulls (legumes). Cell walls have complex structures in which the carbohydrates are intimately associated with non-carbohydrate substances, including vitamins, minerals, trace elements, and bioactive compounds, such as polyphenols and phytosterols), it has different physiological effects and provide a variety of health benefits, including satiety (Slavin, 2007). It is an important concept in preventing weight gain or promoting weight loss. Foods that increase satiety can offset hunger for more extended periods and may reduce the total number of calories a person consumes during a day. Buckwheat is was found rich in polyphenolic compounds, sirtuin regulator functional food in rats on a diet. Functional foods are foods or food components which beyond providing basic nutritional needs, also have physiological/pharmacological benefits or reduce the risk of chronic diseases and also BPA induce stress (Awuchi, Igwe, & Amagwula, 2020). BPA was shown to cause hepatotoxicity as a result of oxidative stress in the liver, which is associated with the development of reactive oxygen species (ROS), which are cytotoxic agents that lead to significant impairment of oxidation (Zeinab K. Hassan, 2012). Buckwheat has been included mainly the Asian and Mediterranean diets and a commonly-eaten food, it is grown in many countries, but Russia is the biggest producer in the world. Polyphenols are the common bioactives in buckwheat, green tea, citrus fruits, richly colored fruits and vegetables, red wines, etc. In yeast, resveratrol (active principle of green tea) express calorie restriction by activating sirtuin, thereby improving DNA stability and enhancing lifespan up to 70%, and it is a proposed activator of sirtuin (Sonia de Pascual-Teresa, 2010). Further investigation revealed that two structurally similar compounds namely quercetin and piceatannol stimulate SIRT1 activity by five- and eightfold, respectively. Both quercetin and piceatannol are polyphenols biochemically. The biological effects of polyphenols are frequently attributed to antioxidant, metal-ion-chelating, and/or free-radical-scavenging activity, and there is a possibility that the stimulation of SIRT1 might simply represent the repair of

oxidative or metal-ion induced damage to the recombinant protein (V. Lobo, 2010). Leptin and adiponectin hormone are found to be beneficially impacted by sirtuin activity. It is noteworthy that these hormones are key regulators of satiety and appetite by regulating activity of hypothalamus (Terence L. Laursen, 2017). Circulating leptin levels illustrates total visceral adipose tissues. As calorie restriction depletes leptin levels, it in turn enhances adiponectin levels, which have been reported to assert a cardio protective effect.

According to some researches the endogenous network of antioxidant enzymes shields the cell against oxidative stress. Ageing signifies accumulation of damaged proteins, lipids, cells, tissues, and organelles in the humans gradually progressing to reduced antioxidant capacity and functionality of these enzymes (Grune, 2015). Consequently, there is an in variant increased ROS production with old age. Because calorie restriction lowers the release of ROS in mitochondria by virtue of sirtuin activation, sirtuin brings about decrease in the detrimental effects of ageing. Buckwheat constituents may positively affect both health and lifespan due to activation of SIRT1 and hence there is a possibility of endothelium protection and NFkB inhibition which is related to SIRT1induction (Chandra K. Singh G. C.-P., 2018). Experimental animals fed diets rich in buckwheat phenolics were reported to exhibit decreased oxidative damage markers such as peroxides in several tissues, and consumption of buckwheat also promoted SIRT1 signaling. Several researches have observed that quercetin which may also exert significant cardioprotective effect by stimulating the activity of sirtuins (Oksana Sytar, 2016). Hence, there are considerable evidences and scientific studies suggesting towards benefits of consumption of specific diet to induce activation of sirtuins, which in turn offers health benefits and promotes well-being.

CHAPTER III

3. Materials and Methods

3.1 Materials

Enzyme-linked Immunosorbent Assay Kit for estimating the protein expression of SIRT1 (Cat# SEE912Ra) was obtained from the Cloud-Clone Corp. (Houston, USA). BPA, Disodium hydrogen phosphate, monosodium dihydrogen phosphate, and sodium chloride were purchased from Medigen, Novosibirsk, Russia. Protein assay kit was procured from Bio-Rad (Hercules, CA, USA).

3.2 Experimental animals

Animal experiments were carried out taking all the required measures to minimize pain and discomfort to the animals by following the standard guidelines laid down by the "National Institutes of Health Guide for the care and use of Laboratory animals" regarding the care and use of animals for experiments and with due approval from the Institutional Animal Ethics Committee headed by Prof. O.V. Smirnova, Chairman of the Committee on Biomedical Ethics, Federal State Budget Scientific Institution "Federal Research Center" Krasnoyarsk Scientific Center of the Siberian Branch of the Russian Academy of Science, Krasnoyarsk, Russia (protocol of the meeting of the expert commission no. 12 from (10) 12- 2018).

3.3 Animal treatment

Male Wistar rats (90-100 grams) were obtained from the Department of Biological Sciences, Siberian Federal University, Krasnoyarsk, Russian Federation. All the animals had free access to drinking water and maintained on 12 hour light and dark cycle (lights switched on at 8 am) with room temperature 22±2°C. Once in the week cages were cleaned and equipped with new bedding. The animals were maintained on different dietary regimes for 8 weeks in individual cages.

3.4 BPA administration

BPA was dissolved in ethanol (1 mg/ml) and administered through drinking water (DW) [16] at the rate of 10mg/L/day. The DW was changed daily and fresh BPA was administered to ensure consistent exposure to the animals and it was particularly ensured that there was no leakage/ spillage of DW from bottles. Based on the measurements of the volume reduction in the DW bottle and assuming that all water lost from the bottle was consumed; the mean levels of BPA consumed by animals was estimated (Table- 1). DW bottles made of polypropylene used for the present study were devoid of BPA and there was no contamination from any sources other than administered DW.

3.4.1 Dietary management

Experimental animals were randomly divided into four groups with 8 rats each namely: A, B, C and D. Each group was administered a specific dietary and DW regimen - **Group A**-Standard rodent pellet (SRP) + NDW (normal drinking water), **Group B**- SRP + BDW (BPA in drinking water), **Group C**- BED (buckwheat enriched diet) + NDW, **Group D**- BED + BDW. The groups of rats administered NDW (group A and C) were given 10mg/L ethanol (vehicle to administer BPA). All the diets and DW were fed *ad-libitum*. SRP were finely crushed while buckwheat was obtained from the local market, crushed and finely powdered in the mill and was then mixed with powered SRP at the rate of 30% level of incorporation. All the diets were stored at 4 °C. The proximate composition of buckwheat in the experimental diet was estimated [17]. The dietary intake and water consumption of the animals was monitored

daily. Body weights of the rats were recorded at the beginning of the experiment and at weekly interval till the end of the experiment.

At the end of the experimental tenure, overnight fasted rats were injected chloral hydrate intraperitoneally (400 mg/kg of body weight) and the hypnotic rats were then sacrificed. Blood was collected by cardiac puncture and allowed to stand for 4 h at 4 °C, followed by centrifugation at 1000 x *g* for 10 min at 4 °C using a table-top Eppendorf centrifuge (5430R, Fisher Scientific, USA). Serum was separated and stored at ─80°C (Sanyo, Ultra-low freezer, Japan) until further use. Liver and stomach were quickly excised, washed with ice-cold saline, blotted dry, and weighed, a small portion (known weight) from them were excised and stored at -80°C until further use.

3.5 SIRT1 estimation

The pre-weighed fresh portions of the tissues (liver and stomach) were homogenized in ice-cold 0.01 mol/L Phosphate Buffered Saline (PBS), pH 7.0 by ultrasonication (Bandelin, Berlin, Germany). The homogenates were filtered using Whatman filter paper No.1 followed by centrifugation at 5000 x *g* for 15 min at 4°C using table top Eppendorf centrifuge (5430R, Fisher Scientific, USA) and the supernatants were carefully collected. These tissue supernatants and serum (collected through centrifugation previously) were used for the determination of SIRT1 protein expression using ELISA. The microplate provided in the kit (sandwich enzyme immunoassay) was pre-coated with a biotin-conjugated antibody specific to SIRT1. The reconstituted standard, samples and subsequent reagents were added to wells. To avoid any cross-contamination, pipette tips were regularly changed between additions of standards, samples, and reagents along with using separate reservoirs for each reagent. Incubation time and temperature were thoroughly controlled and the light sensitive TMB substrate was carefully handled. Avidin conjugated to Horseradish Peroxidase (HRP) was added to each microplate well and incubated. After TMB substrate solution was added, only those wells that contained SIRT1, biotin-conjugated antibody and enzyme-conjugated Avidin exhibited a change in color. The enzyme-substrate reaction was terminated by the addition of H_2SO_4 solution. The color change was measured at Biochrom Annthos 2010 microplate reader at a wavelength of 450nm. The concentration of SIRT1 in the samples was determined by comparing the optical density of the samples to the standard curve. Protein estimation in the tissue extracts were performed using standard Folin-Lowry assay.

3.7 Statistical analysis

The inferences of the present investigation constitute mean ± standard error of mean (SEM) of 8 independent samples. Significance of difference between the mean and one-way analysis of variance (1-way ANOVA) was conducted using Tukey's test. Dependencies were considered statistically significant at the level of significance $p < 0.05$.

CHAPTER IV

4. RESULTS

Widespread contamination and dietary ingestion have led to BPA exposure in the general population, as evidenced by the scientific literature on BPA occurrence in human tissues and body fluids (i.e., urine, serum, plasma, saliva, breast milk, semen, follicular fluids, and adipose tissues) (Meli, 2020). Numerous recent studies on rodents show that exposure to BPA in mothers has a negative effect on offspring. It has also been shown that bisphenol analogues in humans and animals are effectively absorbed through the mouth and spread to the reproductive system, even in the last stages of pregnancy, when they are able to pass through the placental barrier and enter the amniotic fluid (Hidetomo Iwano, 2018). It has been provided much attention to find out the molecular mechanism by which BPA shows its susceptibility to a wide range of disorders, and there is numerous evidence suggests that BPA exposure causes induction of Reactive oxygen species (ROS) and contributes to the predisposition to a variety of toxicity. In a eukaryotic cell, reactive oxygen species (ROS) form as a result of normal physiological conditions in which molecular oxygen is reduced partially. A cells ability to keep balance in the pro-oxidant and antioxidant levels is essential for the normal cellular metabolism, cell survival, and cell proliferation. Several lines of evidence suggest that BPA-induced cytotoxicity caused by oxidative stress occurs in both cell culture studies and an rats model.

4.1 SIRT1 protein expression

a) Serum

The results of the present investigation revealed that exposure to BPA through BDW did not affect SIRT1 protein expression in the serum of SRP fed rats as seen in comparison between group A and B ($p < 0.05$) **(Fig.1)**. DI through BED brought about a significant increase (65.4%) ($p < 0.05$) in SIRT1 protein expression in rats drinking NDW (Group A and C). Also, BED fed rats showed a significant improvement in circulating SIRT1 (90%) ($p < 0.05$) countering stress caused by BPA exposure (Group B and D). Also, BED amounted to 24.7% ($p < 0.05$) improvement in the SIRT1 protein expression in rats subjected to BDW (Group C and D).

b) Liver

In the hepatic tissues of experimental animals, the BPA exposure did not affect the SIRT1 protein expression in the rats fed SRP ($p < 0.05$) (Group A and B). The BED increased SIRT1 protein expression (24.4%) ($p < 0.05$) in rats subjected to NDW (Group A and C). DI through BED led to restoration (26.6 %) ($p < 0.05$) of SIRT1 protein expression in the group of rats subjected to BPA exposure (Group B and D), while 17% ($p < 0.05$) improvement was seen in rats subjected to BDW (Group C and D).

C) Stomach

Likewise, the stomach tissues were not affected by BDW in terms of SIRT1 protein expression ($p < 0.05$) (Group A and B). Under NDW fed state, BED improved SIRT1 protein expression (79%) (Group A and C) ($p < 0.05$); while under BDW fed state, BED affected SIRT1 protein expression (Group B and D) by significant 75.4% ($p < 0.05$) increase **(Fig.1)**.

4.2 Body weight

Dose Level of BPA: Affecting the dose level of BPA is highly controversial. Both *in-vitro* and *in-vivo* data also has contradictory health effects of BPA and create problems for regulatory agencies in evaluating the adverse health effect of BPA. We established the rate of 10mg/L/day with liver toxicity endpoint. Based on these doses used in experiments on BPA it is predicted that a variety of BPA doses induced oxidative stress studies says that these doses

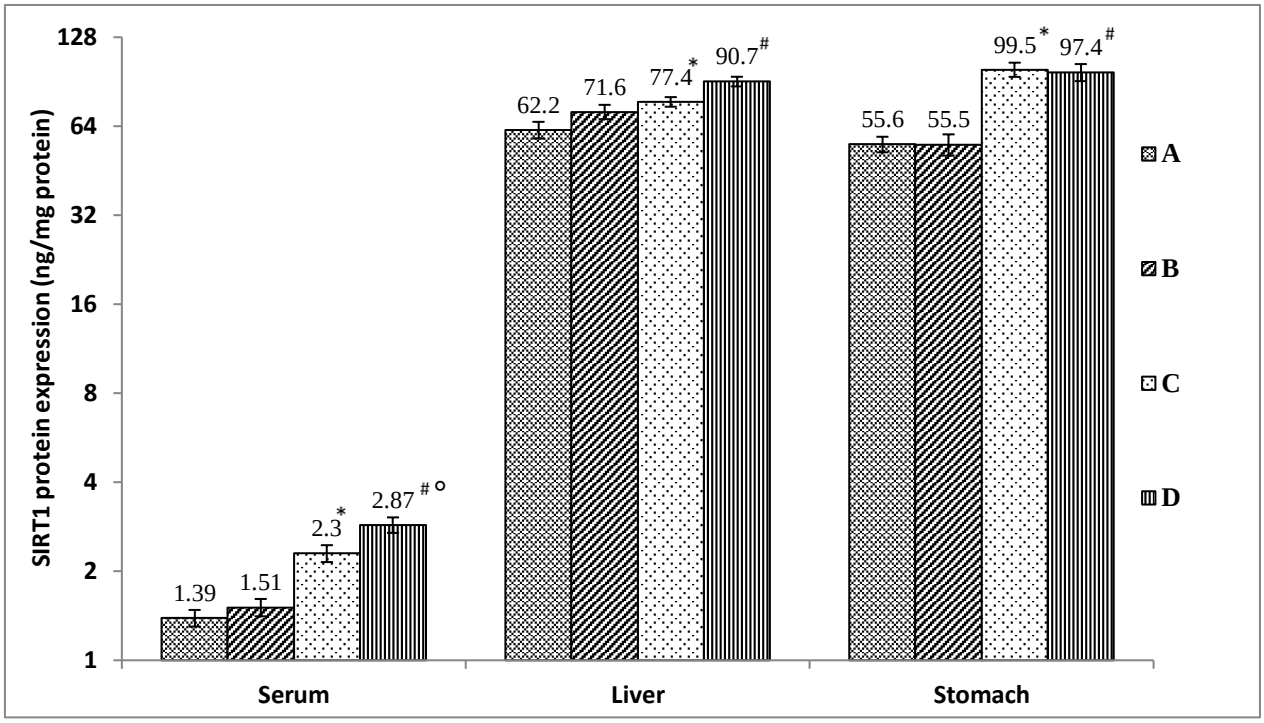
affect differently in inducing oxidative stress. Exposure to BPA or intervention by dietary buckwheat did not affect body/ organ weights during the experiment **(Table-2)**

Table- 2 Effect of BED on absolute and relative (g/100 g body weight) organ weights of rats

Group	Initial body weight (g)	Final body Weight (g)	Gain in body Weight (g)	Organ weights			
				Liver		Stomach	
				Absolute (g)	Relative (g)	Absolute (g)	Relative (g)
A	90.1± 2.12	167.2± 7.81	77.0±3.11	8.83±0.50	5.28±0.43	1.53±0.09	0.91±0.02
B	88.0± 1.13	162.2± 15.8	74.2±3.06	8.76±0.76	5.40±0.11	1.18±0.04	0.72±0.06
C	85.9± 1.0	181.2± 10.9	95.2±2.14	8.65±0.46	4.77±0.32	1.51±0.07	0.55±0.01
D	85.9±2.71	147.4± 10.6	61.5±4.22	7.20±0.43	4.89±0.35	1.48±0.06	1.04±0.03

A- SRP + NDW, **B**- SRP + BDW, **C**- BED + NDW, **D**- BED + BDW (Values are mean ± SEM of eight animals per group)

Fig.1 Impact of BPA and BW on SIRT1 protein expression in different tissues



A- SRP + NDW, **B**- SRP + BDW, **C**- BED + NDW, **D**- BED + BDW

Values are mean ± SEM of eight animals per group.

*significantly different from Group A,

significantly different from Group B,

° significantly different from Group C.

CHAPTER V

5. DISCUSSION

BPA is one of the most commonly known endocrine toxicant released by polycarbonate plastics, lining of food cans and dental sealants. Humans are repeatedly exposed to BPA owing to its extensive availability in the environment. It was demonstrated that BPA induces oxidative stress and seems to play an important role in many degenerative disorders (Manu Rathee, 2012). Perturbation of mitochondrial respiratory chain and induction of oxidative stress are considered to be the major factors leading to mitochondrial injury. It is clear that the mitochondrial respiratory chain dysfunction significantly contributes to tissue injury. We observed that activities of enzyme of ETC complexes were significantly decreased in BPA-treated rat liver mitochondria. Enzymes have been shown to be involved in mitochondrial ROS generation by forward and reverse electron transport, although the mechanism is not fully understood (Dmitry B. Zorov, 2014). The decreased activity of enzymes caused by BPA treatment might have induced ROS production in mitochondria. Oxidative phosphorylation and mitochondrial respiration are mostly regulated by them. A significant decrease in mitochondrial enzymes activity may decrease cardiolipin content (Jana Hroudová and Zdeněk Fišar, 2013). Cardiolipin is located within the inner mitochondrial membrane. Increased superoxide generation in BPA-treated rat liver mitochondria might cause peroxidation of cardiolipin, which might trigger apoptosis with impaired mitochondrial respiration. Alteration in the activities this enzyme complex by BPA may result in disturbance in the flow of electron through various electron carriers in the ETC. Since BPA significantly inhibited the activity of complex enzyme, it may cause impaired energy metabolism. The consequences of toxic effect of BPA on rat liver mitochondria were also reflected in reduced complex enzyme activity. Mitochondrial ATPase catalyzes the final steps of oxidative phosphorylation. Altered ATPase activity may indicate altered cellular functions and may lead to apoptosis. Mitochondrial glutathione is extremely important for regulation of numerous functions. It quenches the free radicals. Observations have shown that there was a significant decrease in the level of GSH in the liver mitochondria from BPA-treated rats (Paul∗, 2019). The decreased (reduced glutathione) GSH level indicates its use in detoxifying the free radicals as shown by increased production of superoxides in BPA-treated rats. Decrease in GSH levels coupled with decrease in the activities of antioxidant enzymes may significantly stress the mitochondria. Superoxide dismutases (SOD) are a class of enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide. As such, they are an important antioxidant defense in nearly all cells exposed to oxygen. The decreased SOD activity suggests impaired dismutation of superoxide radicals resulting in increased hydrogen peroxide (H_2O_2) level (Tohru Fukai, 2011). A significant decrease in (glutathione peroxidase)GPx activity caused by BPA may contribute to oxidative stress in liver mitochondria. Similarly, a decrease in SOD activity has been shown to increase the level of superoxide anion which is known to inactivate GPx. This scenario aggravates the perturbation of mitochondrial bioenergetics. BPA not only causes oxidative stress but also disturbs the balance of antioxidant-pro-oxidant status in the liver mitochondria. Oxidation of GSH builds a pro-oxidative status causing formation of hydroxyl radicals, thereby leading to oxidative damage to mitochondrial membrane. BPA treatment caused a significant increase in serum LDH level. When cells are damaged due to the action of free radicals or when there is oxygen and glucose deprivation, permeabilization of membrane occurs which causes leakage of LDH into the serum. The increase in serum LDH activity could be an indication of tissue damage (Francis Ka-Ming Chan, 2013). Increase in the activities of transaminases indicates liver damage that may occur due to the formation of ROS and other reactive intermediates. Observations of liver mitochondrial dysfunction, oxidative stress, and increased serum hepatic

biomarkers were corroborated with histological findings of the liver of animals exposed to BPA (Bhat*, 2015). Liver injury can result in major blood loss, which could be due to rupture of blood vessels. BPA caused hemorrhage which might be due to injury to blood vessels by its oxidative stress-inducing effect. BPA also caused marked degeneration of hepatocytes, thus causing hepatocellular degeneration (Abdel-Wahab, 2014). Histopathological observations implicitly show role of mitochondrial dysfunction in BPA-induced hepatotoxicity. It has been earlier reported that death of hepatocytes leads to necrosis which could lead cell to apoptosis. BPA also caused liver toxicity as indicated by increase in the activities of serum marker enzymes and histological changes.

The free form of BPA in biological samples is of concern because animal and human studies have identified adverse health effects, many of these reported effects on neurodevelopment, male and female reproductive systems alterations, metabolic diseases and oxidative stress. Studies demonstrated that BPA induced oxidative stress in the liver of rats by decreasing antioxidant enzymes and increasing hydrogen peroxide and lipid peroxidation and co-administration of antioxidant vitamin C reversed this BPA-induced oxidative stress (Maria Elisabeth Street, 2018). In the body, BPA also processed enzymatically by cytochrome P450, and as similar to natural estrogen metabolism BPA converted into quinone form that is reactive to DNA that is lethal to the viability of eukaryotic cells. BPA is structurally similar to estradiol and thus interferes with steroid signaling with different possible outcomes on reproductive health depending on doses, life stage, mode, and timing of exposure (Miki Nakajima, 2005). BPA exerts its epigenetic effects in both male and female reproductive system. In males, BPA affects spermatogenesis and sperm quality and possible trans-generational effects on the reproductive ability of the offspring. In females, BPA affects ovary, embryo and gamete development. It is now investigated that BPA induced oxidative stress as a result of an imbalance between oxidants and antioxidants in the semen can lead to sperm damage, mitochondrial dysfunction, impairments of the structure and function of spermatozoa eventually lead to male infertility. Some studies was based on the findings that sirtuins were NAD^+-dependent protein deacetylases and known to counter aging in yeast. Now, years later, a large volume of data, particularly from mammals, begins to illustrate an elaborate set of physiological adaptations to caloric intake mediated by sirtuins (SINCLAIR1, 2007). Studies that connect sirtuin activation with prevention of aging and diseases of aging in mouse models are many. It is also clear that other nutrient sensors, such as AMPK, mTOR, and FOXO, are very important in linking diet, metabolism, and aging. In addition, it will be critical to learn whether activating compounds affect all SIRT1 substrates or only a subset, as suggested by recent biochemical studies (Picca A, 2017). One might posit that small molecules that bind to the SIRT1 allosteric site mimic natural endogenous compounds that regulate the enzyme under certain physiological conditions; e.g., CR. If so, then the spectrum of effects elicited by the drugs might mimic the effects triggered by these physiological conditions and elicit a coordinated, protective response. Finally, supplementation with the NAD precursors NMN or nicotinamide riboside has been shown to counteract aging and may offer another strategy of keying sirtuin surveillance to forestall aging and degenerative diseases (Christopher R. Martens, 2018). As SIRT1 lacks a DNA-binding domain, it is subjected to target promoters by sequence specific transcription factors so as to incorporate chromatin remodeling and subsequently regulation of gene expression. Also, SIRT1 is associated with the heterochromatin regions where they promote deacetylation of histone (Danny Reinberg, 2004). It can be inferred that owing to multiple molecular functions performed by SIRT1namely deacetylation, epigenetic modifications, and transcription factor modulation; SIRT1 can be potential link between cellular metabolic status and adaptive transcriptional responses (Tong Zhang1 and W. Lee Kraus1, 2010). It is already established that SIRT1 proteins exert their effects through two different pathways namely histone modifications and non histone substrates (Olivier Binda, 2016). In histone modification, many ageing related effects are caused by chromatin changes. Because

SIRT1 is localized mainly in the nucleus, its physiological actions are partly mediated by its ability to deacetylate nucleosomal histones at specific residues. While the nonhistone modification exhibits that once SIRT1 is activated it mediates intracellular responses that promote cell survival, enhance the repair of damaged DNA, and reduce cell division. Experiments indicated that for every acetyl lysine group that is removed, one molecule of NADC is cleaved, nicotinamide and O-acetyl-ADP-ribose are produced (ORCID ProfileHuoqun Gan*, 2020). Therefore, SIRT1 appears to possess two enzymatic activities; first the deacetylation of a target protein and second the metabolism of NADC. This distribution helps us to under-stand the underlying mechanism attributed to sirtuin and its effect on health. In all living organisms, cellular energy is produced and expended using universal "energy currencies" such as ATP and NADH (Guarente2, 2015). The tight balance between such anabolic and catabolic pathways ensures that cells do not deplete essential energy stores, which would ultimately cause cellular damage or death. Sirtuins are significantly involved in mammalian energy homeostasis. The activity of SIRT1 is highly regulated towards the environmental factors that may influence it. A feeding regime that is reported to accelerate the sirtuin activation is caloric restriction, which corresponds to a lowering of 20–40% calories below ad libitum intake without inducing malnutrition (Guarente1, 2013). The levels of sirtuin have been reported to enhance in fasting rats. Much difference in opinion has been reported in context to understand how calorie restriction can affect the life span. It may be a simple analogy that nutrient shortage leads to increased NADC levels, improving sirtuin activity. This shows how calorie restriction improves sirtuin activity. Sirtuins are obviously nutrient sensors as it is actually the nutrient availability which affects the ratio of NADH to NADC. Increase in this ratio will automatically increase sirtuins levels (Dang, 2014). They have already established that the calorie restriction results in an increase in [NADH]/[NADC] levels resulting in increased sirtuin levels, thereby impacting the longevity. Some researchers gave a concomitant report that genetic and pharmacological restoration of NADC levels not only enhances sirtuin activity in experimental animals but consequently delays the ageing process (Auwerx, 2013). In fact, sirtuins were initially identified as mere anti-ageing proteins but at present they have been investigated to be the key agents in providing health benefits through calorie restriction. Ageing has been described to be characterized by declining NADC and delinking PGC-1a/b from mitochondrial control (Rozalyn Anderson1, 2009).

Buckwheat contains dietary fiber which is not digested by rats(also humans). Dietary fibre is a type of carbohydrate that cannot be digested by our bodies' enzymes. It is found in edible plant foods such as cereals, fruits, vegetables, dried peas, nuts, lentils, grains and buckwheat. Indigestible dietary fibers mimic CR in improving of SIRT1 protein expression. BPA induce stress, ROS levels can increase dramatically. This may result in significant damage to cell structures. Cumulatively, this is known as oxidative stress. Effects of ROS on cell metabolism are well documented in a variety of species. These include not only roles in apoptosis (programmed cell death) but also positive effects such as the induction of host defense genes and mobilization of ion transport systems. AMPK, a stress and energy sensor that is activated during low energy states to maintain glucose, fatty acids, and energy homeostasis, is also upregulated in rats treated with SIRT1 activators. The activation of AMPK by SIR1720 is an indirect effect, since SIR1720 does not activate AMPK in rats via acute treatment (Benoit Viollet1, 2011). Rather, SIRT1 activation leads to increased energy expenditure, and the resulting energy-deficit state, reflected by decreased ATP and ADP levels, leads to AMPK activation. Activated AMPK can further amplify the increase in fatty acid usage and mitochondrial capacity (Neil B. Ruderman, 2010). These mechanistic insights on the actions of SIRT1 activators shed light on how the interplay of CR and SIRT1 regulates energy balance at the molecular level. It has been observed that mild oxidative stress conditions induce the expression of SIRT1, changing its activity and thus affecting SIRT1 targets that are involved in the response to changes in the redox state of the cell. The first major SIRT1 substrate identified

was p53, a transcription factor involved in activating antioxidant genes like SOD2 (superoxide dismutase 2, MnSOD) and GPx1 (glutathione peroxidase) (Leonardo Santos, 2016). Another redox transcription factor deacetylated by SIRT1 (as well as SIRT2 and SIRT3) is FOXO3a which induces an antioxidant response via SOD2 and catalase expression. PGC1α, a known substrate of SIRT1, is reported to regulate expression of mitochondrial antioxidants like SOD2. SIRT1 can deacetylate p65 NFκB subunit diminishing its activity and, thus, the production of pro inflammatory cytokines. In addition, upon increased production of ROS at the mitochondria, induction of SIRT3 was observed (Raju, 2015). On the contrary, exposure to high levels of H_2O_2 or harsh oxidative stress resulted in increased proteasomal degradation of SIRT1, desumoylation, and enzyme inactivation that leads to apoptosis (Leonardo Santos, 2016). According to some other researches active sirtuins provide an adequate level of *O*-acetyl-ADP-ribose (OAADPR) (product of the reaction catalyzed by sirtuins with deacetylase activity, that readily converts to ADP-ribose and both may function as cellular signals (Tong L, 2010). Increased ADPR/OAADPR levels protect cells from oxidative stress via two mechanisms: (1) inhibition of Complex I of the mitochondrial electron transport chain with concomitant lower production of ROS and (2) inhibition of glyceraldehyde-3-phosphate dehydrogenase, central enzyme in glycolysis, diverting glucose to the pentose phosphate pathway with the concomitant increase in NADPH, main reductant for detoxifying ROS enzymes.

Buckwheat poly-phenolic compounds reduce ROS levels resulting from reactions with their free radicals. By lowering the level of ROS, they inhibit the initiation of a number of inflammatory processes. To date, the most studied of the SIRT1 activators that antagonize toxic damage is polyphenols. They can enhance the protein expression and activity of SIRTl and binds more easily to substrates following a change in the conformation of SIRTl. Polyphenols can upregulate SIRTl and inhibit the production of reactive oxygen species through the SIRTl/FOXO3 pathway to resist oxidative damage (Zhihua Ren #. H., 2019). They can also regulate heme oxygenase 1 (HO-1) expression through the Nrf2/ARE signaling pathway to protect from oxidative stress damage (Kai Li, 2011). In lead-induced toxic injury, polyphenols increase the level of SIRTl to deacetylate PGC-1a, increase the content of PGC-1a, activate the function of PGC-1a as an NRF-1 co-activator, bind DNA with NRF-1, enhance transcription and activate oxidative phosphorylation reactions (Sergio Rius-Pérez, 2020). In addition, polyphenols also activate SIRT1 and increase MnSOD resistance to lead oxidative stress damage through mitochondrial biogenesis. They can reduce the level of injury through multiple pathways, including inhibiting apoptosis, anti-oxidation, and protecting endothelial cells, and can upregulate SIRTl and reduce the subsequent production of inflammatory cytokines. Furthermore, buckwheat lead to greater satiety due to viscosity of dietary fiber coupled with intake of fewer calories. Satiety is the feeling of fullness after a meal. It is an important concept in preventing weight gain or promoting weight loss. Foods that increase satiety can offset hunger for more extended periods and may reduce the total number of calories a person consumes during a day. The satiety responses from buckwheat flour or groats were measured. The highest satiety score was found with boiled buckwheat groats (Hugo Palafox-Carlos, 2011) (J. Slavin*†, 2007) It is a rich source of protein, so animals did not lose organ/body weight. In experiment all the diets and drinking water were fed *ad-libitum.*

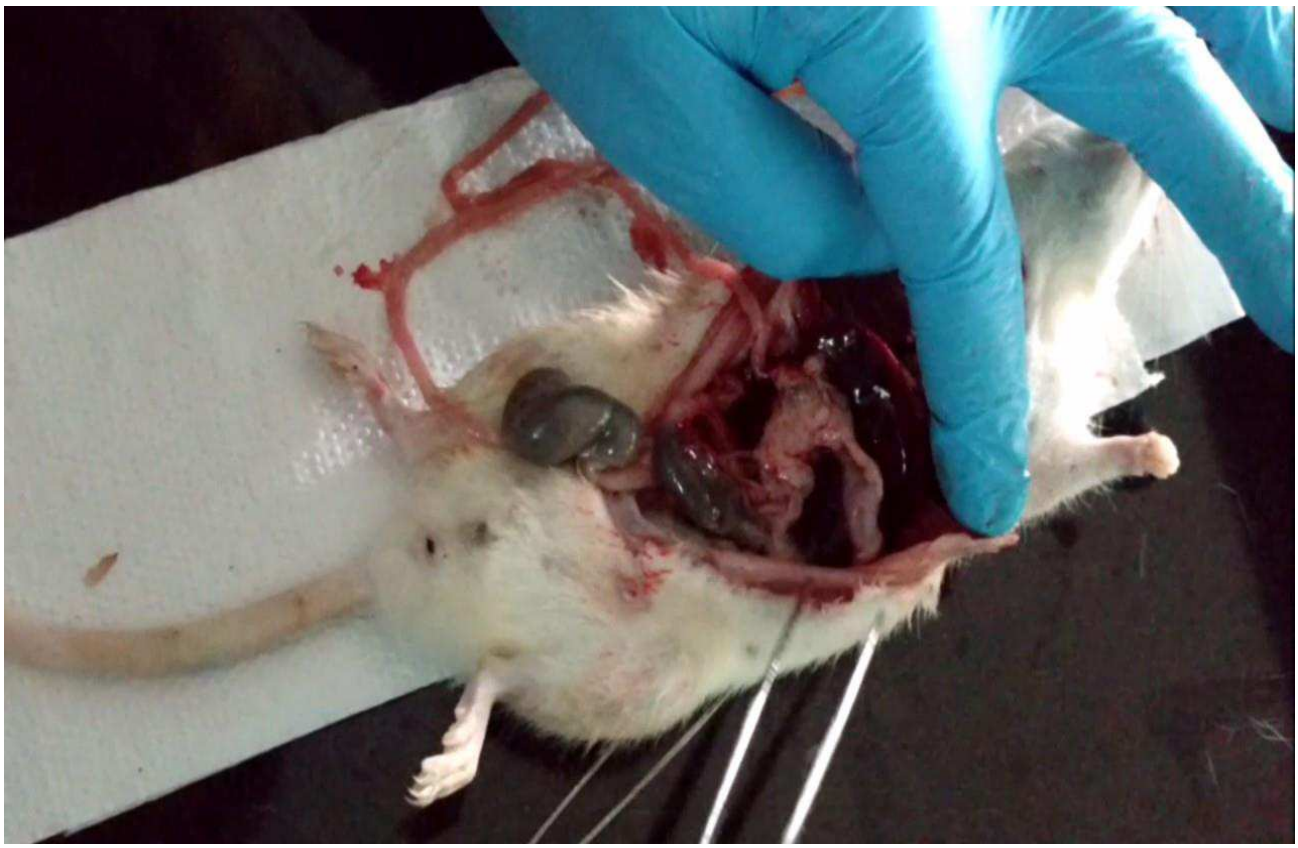
VI. CONCLUSION

It has been known BPA has a harmful effect on health. Overall, BPA exposure significantly decreased activities of enzymes of liver mitochondrial respiratory chain complexes. Alteration in enzyme activities may cause mitochondrial dysfunction, increased ROS generation, and impaired energy metabolism. All these factors may be contributing to BPA-induced hepatotoxicity. The interaction of diet and nutrient-sensing pathways plays an important role in regulating mammalian physiology and health. This review focused on the sirtuins and CR to revisit the original hypothesis that nutrient-sensing regulators mediate the effects of this diet on BPA induce stress. We concluded that dietary buckwheat is effective because it mimics CR, which in turn improves the amount of SIRT1 protein in rats. Experienced animals rich in buckwheat phenolics have been shown to reduce signs of oxidative damage, such as peroxide, in several tissues, and buckwheat consumption has been shown to signal SIRT1. Thus, based on the evidence from our experience about the benefits of consuming a special diet that leads to the activation of sirtuins, we found that it in turn provides health benefits and well-being. Eating plentiful of buckwheat in diet. It can be consumed boiled or as porridge, in soups. All the plant based foods provide dietary fiber (that will work on the same principal as buckwheat in the present study) to improve SIRT1 protein expression.

APPENDIX

1. The animals were maintained on different dietary regimes for 8 weeks in individual cages.

2. Rats were injected chloral hydrate intraperitoneally (400 mg/kg of body weight)



3. The hypnotic rats were sacrificed.

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CONFIRM

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MASTER'S THESIS

The protective effect of dietary buckwheat under the stress caused by bisphenol A

06.04.01 Biology
06.04.01.10 Biological Engineering

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Заведующий кафедрой

_____/В.А. Кратасюк/

«24» июня 2020 г.

МАГИСТЕРСКАЯ ДИССЕРТАЦИЯ

Защитное действие диетической гречки при стрессе, вызванном бисфенолом А

06.04.01 Биология
06.04.01.10 Биологическая инженерия

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