A Review of the Effects of Blueberry on Obesity, Cancer and Neurocognitive Decline

Farideh Shafiee-Kermani and Mihai D Niculescu*
1USDA-ARS, North Carolina Research Campus, USA
2Department of Nutrition, University of North Carolina at Chapel Hill, and UNC Nutrition Research Institute, USA

*Corresponding Author: Mihai D Niculescu, Department of Nutrition, University of North Carolina at Chapel Hill, and UNC Nutrition Research Institute, 500 Laureate Way, Kannapolis, NC 28081, USA.

Received: February 17, 2015; Published: April 24, 2015

Abstract

Studies utilizing plant extracts have demonstrated some protective effects against degenerative, chronic diseases such as cancer and cognitive impairment and other health problems such as obesity. This protective effect has been attributed to their phenolic contents. Research suggests oxidative stress and cellular immunity dysfunction as the contributors in the pathogenesis of these diseases. Blueberries are particularly rich in polyphenols and have shown to have antioxidant, anti-inflammatory responses. In recent years, these berries have received attention due to their possible potential for ameliorating these diseases. Most of these studies, especially in the case of cancer, are based on in vitro examinations. The animal and clinical studies are limited and not always supportive in all these cases. This review focuses on the studies exploring the effect of blueberries on obesity, cancer, and age related neurodegenerative disorders. It also discusses some inconsistencies that exist among the published results and recommends research plans that might help to better determine the beneficiary effects of blueberry interventions on these diseases.

Keywords: Blueberry; Obesity; Cancer; Neurocognitive decline

Introduction

Dietary consumption of berry fruits has been shown to have a positive impact on human health and performance [1]. Studies utilizing plant extracts have demonstrated protective effects against many degenerative and chronic disorders [2-7]. These protective effects have been attributed to their phenolic contents [4,8]. Although the precise mechanisms underlying the initiation and progression of these disorders are not completely understood, research suggests oxidative stress, cellular immunity dysfunction, and alterations in cellular signaling pathways, as contributors to their pathogenesis [1,9-11]. Because many of the existing drugs often have strong side effects and are not targeted to preventing the underlying mechanisms responsible for these complex conditions, a strong interest exists in developing food-based, dietary approaches that may hold promise as effective and safe interventions against these complex disorders [12-13].

Polyphenols are metabolites in plants and to date, more than 8000 phenolic compounds have been identified in human diets. The major classes of dietary polyphenols are phenolic acids, stilbenes, curcuminoids, and flavonoids. Phenolic acids and flavonoids account for 30% to 60%, of dietary polyphenols, respectively [14]. Blueberries belong to Vaccinium genus and are native to Northern America [15]. They are particularly rich in phenolic acids and flavonoids such as hydroxycinnamic acids and proanthocyanidines/anthocyanins, respectively, in addition to containing significant amounts of micronutrients and fiber [16-18]. In vitro, blueberries have shown to have strong antioxidant activity, comparable with that of blackberries, strawberries and cranberries [19]. In recent years, blueberries have received increasing attention due to their possible potentials for the prevention and/or reversal of cognitive impairment [20], ischemic heart disease [21], obesity [16], and cancer [22]. Although promising, most of these studies, especially in the case of cancer, are based on

in vivo examinations. Animal and clinical studies are limited and not always supportive in all these cases and there is no epidemiological research available yet. In this review we focus on the present knowledge regarding the impact of blueberry on obesity, cancer, and cognitive decline, and view some inconsistencies that exist in the reported data. We also sketch and recommend research plans that might help to better evaluate the potential beneficiary effects of blueberries upon human health.

**Obesity**

Obesity is a serious health issue in the developed countries and is becoming increasingly alarming on a global scale. It is characterized by hyperlipidemia and insulin resistance that commonly precedes the development of type 2 diabetes and heart disease [23]. The results of in vitro, in vivo, and the limited clinical studies, discussed here, show that blueberry extracts can reduce or mitigate molecular markers of obesity, but may not be effective in inducing weight loss.

**In vitro studies**: Using a cell line capable of differentiating into adipocytes, 150-250 µg/ml of blueberry freeze dried powder, altered adipogenesis by suppressing adipocyte differentiation and proliferation. However, lipolysis was unaffected by blueberry treatment in these cells [24]. This discrepancy could be the result of low sensitivity of available assays for detection of free fatty acids. In another in vitro study using cultured insulin sensitive muscle cells and adipocytes, it was shown that 30 µM fermented blueberry juice (has 4 times more antioxidant activity than unfermented), but not unfermented, increased glucose transport, which was further augmented when combined with insulin. The juice also decreased triglyceride accumulation and increased the activity of AMPK (adenosine monophosphate-activated protein kinase), a protein involved in insulin independent pathway of lipid metabolism, while failed to alter the activity of insulin-dependent kinases involved in glucose metabolism, Akt (protein kinase B) and/or ERK1/2 (extracellular-signal-regulated kinases). It also failed to stimulate adipogenesis [25]. These data suggested a link between the antioxidant effect of fermented blueberry juice and the modulation of glucose and lipid homeostasis by an insulin-independent mechanism.

**In vivo studies**: An in vivo studies using a leptin resistant mice, the same fermented blueberry juice [25] (40 ml/kg body weight), when incorporated to drinking water for 3 weeks, protected the pre-diabetic 4 weeks old mice from hyperphagia and reduced their weight gain. It also protected them against developing glucose intolerance and diabetes [25]. In obese, diabetic 7 weeks old mice, administration of 80 ml/kg body weight of the fermented juice for 4 weeks also reduced the food intake, body weight gain, and increased adiponectin levels [26]. Adiponectin has been shown to reduce triglycerides levels and reverse insulin resistance phenotype in obese mice. These data indicated that fermented blueberry juice, which has higher antioxidant capacity, can reduce food intake, body weight gain, and development of diabetes/insulin intolerance in vivo, perhaps by increasing fat metabolism. In opposition, studies using mice, susceptible to diet-induced obesity, consuming a high-fat diet (60% calories from saturated fat) supplemented with 4% blueberry freeze dried powder for 8 weeks, indicated that blueberries were ineffective in attenuating weight gain or adipose tissue weight. However, the supplementations with blueberry powder increased insulin sensitivity and improved glucose homeostasis. These alterations were accompanied by a protective effect against adipocyte death and down-regulation in macrophage gene expression of inflammatory cytokines, TNF-α (tumor necrosis factor-α) and IL-10 (Interleukin-10). The expression of CD11c (integrin), a cell surface marker for macrophages, was also down-regulated, indicating an inhibition of macrophage infiltration [27]. Adipocyte death attracts macrophages resulting in proinflammatory cytokines release and development of a mild chronic inflammation that over time leads to insulin resistance [28]. Blueberry anthocyanins (0.2 mg/ml) dissolved in drinking water or blueberry juice (2.8 ml/mouse/day) for 72 days reduced body fat and serum leptin of obesity prone mice fed a high fat diet (45% calories from fat) to the levels of mice fed a low fat diet (10% calories from fat). Anthocyanins drink, but not blueberry juice, reduced retroperitoneal and epididymal adipose tissue mass, and reduced fasting serum glucose concentrations of mice fed the high fat diet to the levels of mice receiving the low fat diet. Anthocyanins drink also returned β cell function score and insulin release to normal levels in high fat diet mice. These data indicated that purified anthocyanins are more effective than blueberry juice in affecting obesity and its symptoms. [29]. In Zucker Fatty rats fed a high fat diet (45% kcal from fat), the addition of (2% W/W) blueberry freeze dried powder reduced triglycerides, fasting insulin, and insulin resistance [23]. Blueberry intake also reduced abdominal fat mass, increased adipose and skeletal muscle PPARs γ and α (peroxisome proliferator-activated receptors) activity,

and affected transcripts of PPARs and genes involved in fat oxidation and glucose uptake, but did not affect total fat mass or body weight [23]. PPARs are nuclear fatty acid receptors that play an important role in obesity-related metabolic diseases such as hyperlipidemia, insulin resistance, and coronary artery disease [30]. In addition to the above effects, blueberry intake reduced liver weight, body weight, and total fat mass in Zucker Fatty rats fed low-fat (15% kcal from fat) diet [23]. In Zucker Lean rats fed a high-fat diet, the addition of blueberry increased body weight and reduced serum triglycerides, without affecting other parameters. In Zucker Lean rats fed a low-fat diet addition of blueberry did not have any impact on the measured parameters [23]. These data indicated that the effect of blueberries on obesity differs based upon the dietary regimen and perhaps the severity of the obesity symptoms. Studies using OLETF obese rats have shown that addition of hot water fraction (HW), flavonol glycoside (FG), and proanthocyanidin (PA) extracts from blueberry leaves to the diet, for 4 weeks, neither affected food intake nor body, liver, or total fat weight. It also failed to affect serum triglyceride, glucose, insulin, leptin, and MCP-1 (monocyte chemotactic protein 1), a cytokine involved in glucose uptake. However, FG and PA reduced cholesterol levels and CRP (C-reactive protein, a risk factor cytokine for type 2 diabetes and cardiovascular disease). Hepatic triglycerides were reduced by all extracts, but only PA reduced activity of malic enzyme, an important factor for fatty acid synthesis [31-32]. HW and PA also reduced the activity of CPT1 (carnitine palmitoyltransferase I), a key enzyme in mitochondrial fatty acid beta-oxidation. These data suggested that various extracts from blueberry leaves differentially affect lipid metabolism in OLETF obese rats [32].

Clinical Studies: Effects of blueberries on obesity and its parameters in humans have also been studied. A double-blind, randomized, and placebo-controlled clinical study in obese, insulin-resistant men and women reported that the dietary intake of smoothie containing 22.5g blueberry freeze dried powder 2 times/day for 6 weeks significantly improved insulin sensitivity in these subjects who were at high risk for type 2 diabetes. However, the blueberry extract neither affected body weight, adiposity, or energy intake, nor the levels of inflammatory biomarkers, CRP, TNFα, and MCP-1 in the serum of these subjects [33]. Another randomized controlled trial in obese men and women showed that 8 weeks daily consumption of 50 g freeze-dried blueberry powder reconstituted in 480 ml of water significantly decreased systolic and diastolic blood pressure in this group who were susceptible to hypertension. Blueberry extract also decreased levels of biomarkers of lipid and lipoprotein oxidation, ox-LDL (oxidized low density lipoprotein), MDA (malondialdehyde), and HNE (hydroxynonenal) in the plasma of these subjects, while neither affected serum levels of glucose and lipid profiles nor the biomarkers of inflammation, CRP, IL-6 (interlukin-6), and adiponectin. The extract was also ineffective against body weight gain or in reducing the waist circumference [34].

Discussion: Reported studies herein support the beneficial effects of blueberry and their polyphenols, in particular anthocyanins, on alleviating adverse effects of obesity such as chronic inflammation, insulin resistance and impaired glucose and lipid metabolism. However in some cases, especially in humans, it appears that blueberry failed to induce weight loss. One important aspect to be noted in the case of dietary polyphenols is that their bioavailability is far below the ingested concentrations [35], thus, short time exposure to these compounds at older ages may not be sufficient to prevent weight gain or, at best, could cause only small changes that would not be significant in data analysis. This aspect is especially important when the sample size is small, since it can shield the distinct conclusions. Thus, continuous long time interventions that may cause small biological alterations, in which on long term, could significantly alter the obese-related phenotypes might help providing more conclusive results. In fact, it has been shown that events occurring during critical developmental stages influence obesity risk and disease development at a later time in life. Especially, altered maternal nutrition can lead to metabolic disorders in progenies, characterized by obesity [36].

Conclusions: In regard to above concepts, it may be important to design lifelong (from prenatal to older age) studies in animal models for obesity in order to more properly analyze the impact of dietary blueberry consumption on weight gain. However, since conducting life time research in humans is not feasible, well designed epidemiological studies may help verifying these effects.

Cancer

Epidemiological studies have demonstrated that despite advances in cancer therapy, the incidence and cure rate of cancer have not improved significantly since half a century ago [37]. The in vitro data discussed here show that blueberry extracts exert anticancer properties by altering cellular apoptosis, proliferation, differentiation, angiogenesis, metastasis, and inflammation [38,39]. However, not all of the limited in vivo data support the anticancer activity of blueberry.

In vitro studies: Treatment of two breast cancer cell lines, MDA-MB-231 (estrogen non-responsive) and MCF7 (Estrogen responsive) with 250 µg/ml anthocyanin and/or anthocyanin-pyruvic acid adduct extracts from blueberries, for 24h, reduced cell proliferation and invasion [22]. An ethanol fraction extracted from blueberry leaves have shown to inhibit proliferation of 4 different adult T-cell leukemia with an IC<sub>50</sub> of 10 µg/ml after 72h of treatment [40]. Anthocyanins from bog bilberry (a member of the blueberry genus) also decreased cell viability in a hepatocarcinoma cell line (HepG2) with an IC<sub>50</sub> of 0.563 mg/ml and in a colon cancer cell line (Caco-2) with an IC<sub>50</sub> of 0.390 mg/ml. The decrease in viability was accompanied by alterations in cell cycle progression and increased membrane permeability that were evident by increased percent cells in sub-G1 phase and LDH (lactate dehydrogenase) release (a marker of membrane permeability and apoptosis), respectively [41]. Bilberry (myrtle blueberry, native to Europe) extract at concentrations of 0.3-0.4 mg/ml induced apoptosis and inhibited proliferation in MCF7 breast cancer cells. However, it did not affect cell cycle phases or organization of microtubules that are important for mitotic cell division. These data indicate that inhibition of proliferation and/or induction of apoptosis, at these concentrations, are not correlated with the modulation of the cell cycle or alterations in the organization of microtubules. But at higher concentrations (0.5-0.9 mg/ml), however, in addition to the effect on proliferation, accumulation of cells at G2/M stage, the cell rounding, and the accumulation of tubulin in punctate spots occurred. These data indicated that the effect of bilberry extracts on microtubule organization was concentration-specific and correlated with a separate direct action of the extracts that only occurs at higher concentrations [42]. Blueberry polyphenols were separated into phenolic acids, tannins, flavonols, and anthocyanins, and their antiproliferative effect on two colon cancer cell lines, HT-29 and Caco-2, were assessed. Among these fractions, anthocyanins (15-50 µg/ml) had the highest antiproliferative effect with > 50% inhibition. Anthocyanin fraction also resulted in 2-7 times increase in DNA fragmentation that is an indicator of apoptosis. Flavonol and tannin fractions resulted in 50% inhibition of cell proliferation at concentrations of 70-100 and 50-100 µg/ml, respectively. The phenolic acid fraction showed relatively lower bioactivities with 50% inhibition at 1000 µg/ml [43].

These data demonstrated that different classes of polyphenols had different potencies in preventing proliferation. A mixture of the seven phenolic acids from blueberry, namely hippuric acids, suppressed primary mammosphere formation of MDA-MB-231 cells in a dose-dependent manner, at 5 and 10x the concentrations find in the serum of rats fed 10% blueberry, while at highest dose (20x) had no activity relative to control. These phenolic acids did not affect PTEN (Phosphatase and tensin homolog), a tumor suppressor gene, production [44]. These data indicated that the anticancer effects of phenolic acids are highly concentration-specific, and that different blueberry fractions might have different roles in different cancer types or cell lines.

In vivo studies: Research using Fisher 344 male rats showed that diet containing 5% (W/W) blueberry freeze dried powder for 13 weeks significantly reduced azoxymethane-induced aberrant crypt foci (ACF) in colons of these rats. The extract also increased hepatic glutathione-S-transferase activity in these rats, which is an indication for antioxidative properties of blueberry through activation of phase II metabolizing enzymes [45]. In contrast, studies using Sprague-Dawley rats showed that diet containing 10% (W/W) blueberry freeze dried powder for life time (prenatal to the time of experiment) was ineffective in inhibiting azoxymethane-induced ACF in colons of these rats. Blueberries only tended (insignificantly) to reduce the total number of colon ACF and overall intestine tumor incidence in males. In female rats, however, blueberry significantly augmented ACF numbers in distal colon, which is contradictory to its expected beneficial effect in cancer prevention [46]. It was concluded that these differences in outcomes from these two studies may be explained by the concentration-specific effects of the blueberry extract (5% vs. 10%) or the possible genetic background (Fisher 334 vs. Sprague-Dawley rats) variations in response to treatments.

Conclusions: As it appears from the studies presented here, the anticancer activity of blueberries varies depending on the concentration, duration, extract composition, and cell types or the animal strain that have been used. In order to more precisely verify the beneficial impacts of blueberry interventions on prevention and treatment of cancer, it is clearly necessary to design more animal studies using wide range of various concentrations of whole and/or different classes of blueberry extracts. It is especially necessary to conduct long term clinical trials using large number of individuals, which is presently missing, for different types of cancers to better evaluate the effect of blueberry on cancer prevention/treatment.

Neurocognitive Decline

Aging is generally associated with common chronic cognitive decline [51,52]. Oxidative stress and inflammation have been implicated in neuronal degeneration and dementia [53,54]. In vitro and in vivo studies and a single human trial presented support to the positive effect of blueberry on cognitive decline possibly by reducing oxidative stress and inflammation that restores the normal functioning of neuronal cells.
**In vitro studies:** In a study using lipopoly saccharide activated BV2 microglial cells, blueberry polyphenolic extract attenuated production of nitric oxide (NO) and reactive oxygen species (ROS) dose dependently. It also reduced mRNA and protein levels of inflammatory enzymes, INOS (inducible nitric oxide synthase) and COX2 (cyclooxygenase-2), and proinflammatory cytokines (IL-1β and TNF-α) [55]. The following study showed that blueberry polyphenols sequestered nuclear translocation of the transcription factor, NF-κB, which then attenuated transcriptional activation of proinflammatory cytokines [8]. These results suggested a possible beneficiary effect by blueberry polyphenols in protecting neurons in CNS from oxidative and inflammatory insult. A recent study using TNF-α activated SH-SY5Y cells, a human neuroblastoma cell line, demonstrated the obstruction of coalescing of lipid rafts into larger platforms by a blueberry fraction, depleted of polyphenols. This effect was caused by impeding the assembly of NADPH oxidase (NOX2) and, hence, interrupting the association of the cytosolic p67phox with NOX2 and gp21phox in plasma membrane, which resulted in abolishing ROS production. These data indicate that blueberry extracts other than polyphenols, can also affect ROS production by interrupting the assembly of membrane enzymes [56]. It was postulated that the loss of cognitive function in aging may be dependent upon dysregulation in calcium homeostasis. In this regard, blueberry freeze dried water soluble extract antagonized the negative effects caused by stressors, dopamine and amyloid β, on calcium buffering in primary rat hippocampal cells and altered the levels of phosphorylated signaling molecules, MAPK (mitogen-activated protein kinases), PKCy and CREB (cAMP response element-binding protein) [57]. Another study showed that a blueberry polyphenolic enriched fraction prevented neuronal synaptic failure and ATP imbalance induced by amyloid β (Aβ) aggregates in primary hippocampal neurons of wistar rat embryos exposed to soluble oligomers of Aβ. This effect was induced by altering the aggregation state of Aβ, restoring the frequency of synaptic currents, and decreasing the neurotransmitter-containing vesicles. Also, the extract partially antagonized the decrease in intracellular Ca2+ activity and the acute ATP leakage but not chronic ATP depletion [58].

**Combined in vitro/in vivo studies:** In a combined in vitro/in vivo approach, hippocampal regions from young and old rats fed a blueberry supplemented diet for 10 weeks, were subjected to LPS (lipopolysaccharide) inflammatory challenge in vitro and HSP70 (heat shock proteins 70) levels were then measured. The blueberry diet completely restored the HSP70 levels in the hippocampus of old rats to the levels present in young ones. HSP70 has been shown to protect neurons against various insults [59].

**In vivo studies:** studies in aged rats showed that a diet containing 2% blueberry lyophilized powder attenuated age-related object recognition memory impairment and reduced NPx8 levels to the levels of young rats through reduction of aging-induced oxidative stress [60]. The above dietary blueberry supplementation also reversed the effect of kainite-induced cognition and learning impairments in rats [61]. It also preserved neurogenesis in the hippocampus of old rats [62]. Another study in aged rats showed dietary intervention with 2% (W/W) of blueberry lyophilized powder increased spatial memory that was accompanied by elevated CREB activity [63]. CREB activity is pivotal for switching from short-term to long term memory. Blueberry supplementation also increased ERK1/2 activity which is suggested to be important for synaptic plasticity and memory formation. In addition, blueberry increased hippocampal BDNF (Brain-derived neurotrophic factor) and Arc/Arg3.1 (activity-regulated cytoskeleton-associated protein) proteins that are necessary for learning and memory in adults [63]. In another study, old rats that ate a blueberry supplemented diet for 2 months had significantly better balanced walking and performed better on the water maze test than the control rats fed placebo [64]. However, in a similar study in which the rats were given a blueberry supplement for an extra month, they demonstrated improved motor function, but did not have improved cognitive function as measured using the water maze test [20].

**Clinical studies:** A clinical trial investigating the beneficiary roles of consumption of blueberry juice in the treatment of 9 elderly cases (women and men) with mild memory decline and increased risk for dementia showed that 6-9 ml/kg body weight of commercially available blueberry juice, administered once a day for 3 months, significantly improved memory function as determined by two different tests, paired associate tasks and list learning tests. Although not significant, the data showed also a trend towards lower glucose levels along with correction of fasting insulin to the normal range in these subjects that may have suggested an association to alterations in inflammation and greater clearance of central β-amyloid as well as enhanced signaling in memory centers. The primary limitations of this study was the small sample size that could have affected substantial effect size and statistical significance [65].

A Review of the Effects of Blueberry on Obesity, Cancer and Neurocognitive Decline

**Discussion:** As it appears thus far, the use of blueberry in the prevention and treatment of cognitive impairment has been proven promising. *In vitro, in vivo*, and limited human studies have provided evidence that links oxidative stress imbalance and increased inflammation to neurodegeneration and cognitive decline. Blueberry and its phenolic constituents rescued neuronal populations and synaptic connections from deterioration, by reducing production of inflammatory enzymes and cytokines, while restoring the activity of signaling molecules and production of proteins pivotal for synaptic plasticity and restoration of memory. Although most studies point out towards the effects of polyphenols, non-phenolic extracts from blueberries have also been shown to reduce production of reactive oxygen species by penetrating into lipid rafts and, thereby, interrupting the assembly of membrane-bound enzymes in a human neuroblastoma cell line [56].

**Conclusions:** Although the data presented here are generally positive and promising, there is only one data from clinical trial on a small group and for a short intervention period available. To determine blueberry effects on cognitive decline in humans, more studies, especially, long term clinical studies using blueberry interventions in larger groups of patients with cognitive impairments are necessary.

**Overall Discussion**

Although studies presented here generally provide consistent, positive evidence for the beneficiary effects of blueberry on neurocognitive decline and obesity biomarkers mitigation, there are some inconsistencies among the results regarding the effects of blueberries on weight gain and, specially, cancer, which raises concerns when designing experiments. One important consideration is the optimum age and duration of intervention. The complex molecular alterations leading to initiation, progression, and manifestation of chronic, degenerative disorders in humans may occur years before the onset of the clinical symptoms. In fact, more recent studies have shown that the potential and molecular predispositions of these diseases are, at least in part, determined prenatally during embryonic development and are greatly affected by maternal nutritional and health status [66]. Furthermore, it has been shown that these molecular alterations can be passed on to the future generations [67]. Additionally, as it appears from animal and cell culture studies, the effect of blueberry extracts are highly concentration dependent.

**Overall conclusions**

It is obvious that more long-term animal studies that cover, at least, one complete whole life exposure (pre and post natal) to various concentrations of dietary blueberry extracts are needed in order to more precisely determine the health benefits of blueberry interventions in research animals that may mimic the effect in human. Although designing long term studies are feasible in rodents (2-3 years), it is not attainable in humans. In addition, since consumers usually rely on oral dietary consumption of fruits, it may be helpful to conduct epidemiological studies that cover a large number of subjects selected from areas where people traditionally eat high and/or low volumes of blueberry in order to better verify the beneficiary impact of this fruit on the above biological chronic dysfunctions in humans. The more attainable alternative to population studies could be long term clinical interventions using large groups of individuals and when enough numbers become available, meta-analysis can be employed to provide scientific evidence. These may help to better analyze the blueberry effects in humans. Finally, without more clinical and/or epidemiological studies, it is difficult to draw a clear conclusion regarding the beneficial effects of blueberry on human health.

**Bibliography**


**Citation:** Farideh Shafiee-Kermani and Mihai D Niculescu."A Review of the Effects of Blueberry on Obesity, Cancer and Neurocognitive Decline", *EC Nutrition* 1.3 (2015): 145-154.
A Review of the Effects of Blueberry on Obesity, Cancer and Neurocognitive Decline


A Review of the Effects of Blueberry on Obesity, Cancer and Neurocognitive Decline


**Volume 1 Issue 3 April 2015**
© All rights are reserved by Mihai D Niculescu, *et al.*