Chapter 1

Review of related literature and studies

1.1 CARDIOVASCULAR DISEASE

Cardiovascular diseases (CVDs) are the major cause of death in India and worldwide. In 2015, CVDs accounted for one-third of all deaths worldwide. CVDs are expected to be the fastest growing chronic illnesses, which is increasing at the rate of 9.2% annually, and accounting for the second largest number of non-communicable disease (NCD) after mental illnesses. A more worrying fact is that the incidences of CVDs have gone up significantly for people between the age 25 and 69, which means we are losing more ‘productive’ people to this disease (Organization, 2011; Shokeen & Aeri, 2015). The load of communicable to non-communicable disease is projected to get reversed in 2020 as compared to its distribution in 1990. This is primarily because large section of the population has moved towards unhealthy lifestyles with decreasing physical activity, increasing stress levels, increasing intake of saturated fats, consumption of tobacco and related products (Jogsan, 2014; Shokeen & Aeri, 2015) which disturbs the fine balance between free radicals and various endogenous antioxidants in favor of free radicals.

Over 300 risk factors alone or in combination have been associated with CVDs like non-modifiable risk factors (advancing age, male gender, family history/genotype), metabolic risk factors (hypertension, hyperlipidemia, diabetes mellitus, metabolic syndrome, obesity/overweight), life style (diet, smoking, physical activity) and novel risk factors (elevated homocysteine level, elevated lipoprotein-A level, small dense LDL-C, elevated inflammatory markers levels, elevated hemostatic factors levels). Among these, lifestyle is modifiable risk factor which can be improved to prevent the further progression of CVDs (Alissa & Ferns, 2012).

Pathological conditions

***Atherosclerosis*:**

The prolonged pathogenesis of atherosclerosis is marked by endothelial cell dysfunction, followed by deposition of lipid-laden macrophages and vascular smooth muscle cell proliferation, leading to occlusion of the arterial lumen. Hypertension and hyperlipidemia significantly contribute to the process of atherosclerotic plaque formation (Singh et al., 2002).

***Cardiac ischemia*:**

Cardaic ischemia is the cause of several risk factors which results into irreversible cell damage. Atherosclerotic plaque formation, clinical situations of angina, coronary vasospasm, and balloon angioplasty are the reasons for myocardial ischemia, which normally myocardium can tolerate up to 15 min but with increase in duration and severity ischemia induces cardiomyocyte damage (Rodriguez, Trayanova, & Noble, 2006).

These pathological conditions have always been targeted to reduce deaths due to CVDs by reducing the numerous pathophysiology and biochemical changes such as oxidative stress, lipid peroxidation, hyperglycemia, hyperlipidemia, etc.

Factors affecting cardiac output

Cardiac output is regulated by stroke volume and heart rate. Preload (effect of stretching), contractility and afterload are the factor involved in stroke volume regulation. Preload regulates the blood volume in ventricles, contractility defines the strength of contraction at any given preload and afterload prevents the opening of semilunar valve by overcoming the right and left ventricular pressure. Thus, increase in afterload can deteriorates the normal blood circulation. Also, Heart rate plays an important role in short term control of cardiac output and blood pressure. Cardiovascular centre in medulla oblongata regulates the heart rate by receiving inputs from sensory receptors and from higher brain centers. Propioceptor, chemoreceptor, baroreceptor and sympathetic neurons senses a signal from change in position of limbs and muscles; chemical change in blood; stretching in arteries and veins and from cardiac accelerator nerve activated β1adrenergic receptor (AR) to induce the necessary changes in heart rate. There are several other factors like hormones, ions, temperature, sex and age which regulate the heart rate. Imbalance in all these factors leads to trachycardia, bradycardia or hypertrophy (Tortora & Derrickson, 2008).

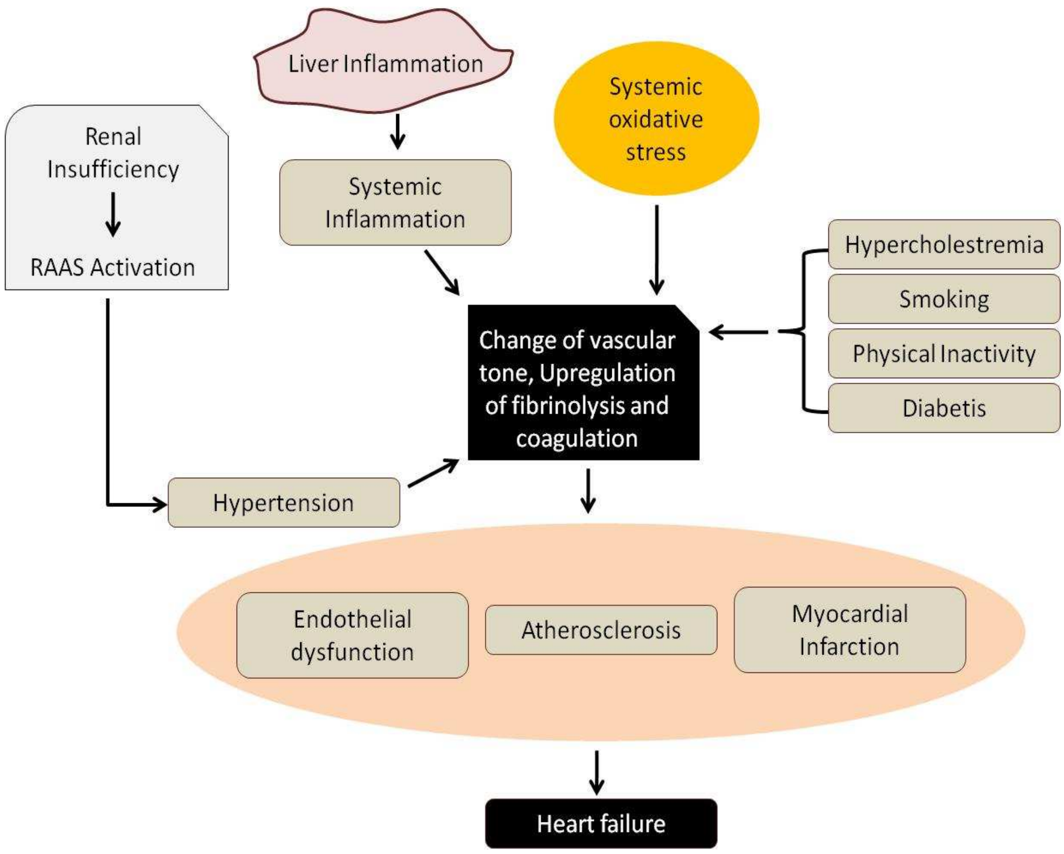


Figure 1.1: Schematic representation of factors involved in progression of cardiac disease. Flow chart represents the role of inflammation, oxidative stress, RAAS activation and lifestyle in CVDs.

Clinical strategies and outcome

Coronary artery bypass grafting and percutaneous coronary intervention are more often scrutinized for ischemia reperfusion related abnormalities in the post-operative recovery of the patient, showing the need for the use of therapeutic strategies to prevent the post-operative ischemia. Several therapeutic strategies showed promising results in clinical studies and still some clinical studies are in progress to evident the therapeutic strategies against post-operative ischemia.

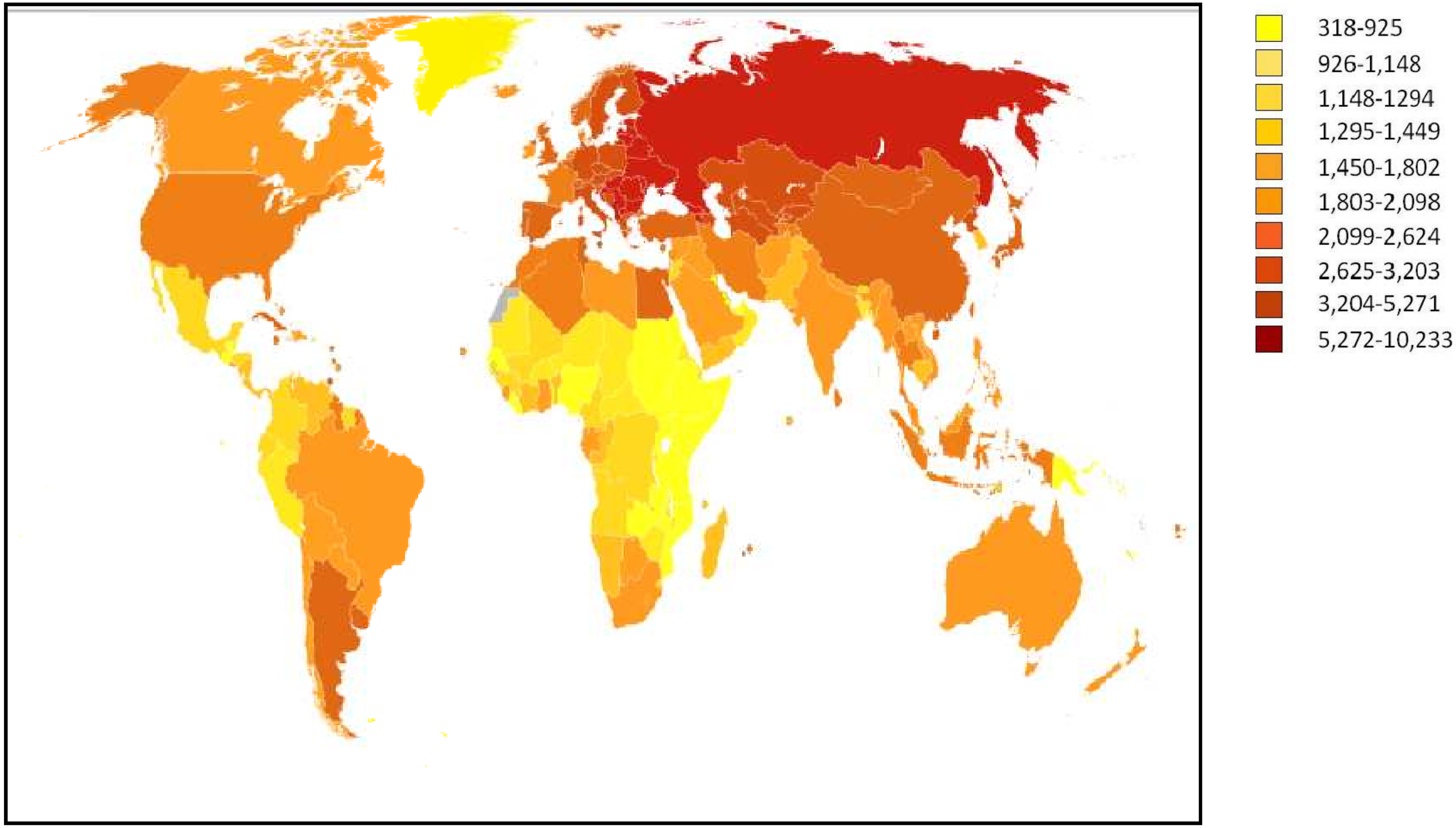


Figure 1.2: WHO report on death due to CVDs

Reference: https://Cardiovascular_diseases_world_map-Deaths_per_million_persons-WHO2012.svg

1.2 REPORTS ON DIETARY FACTOR IMPROVING CVDs

Dietary factor play a key role in the development and improvement of various human diseases, including cardiovascular disease. There has been increasing recognition that certain natural substances have potential to reduce the detrimental effect of cardiovascular risk factors. Epidemiological studies have shown that diet rich in fruits (Jadeja et al., 2010; Lopera et al., 2013), herbs (Hsieh et al., 2014; Patel et al., 2012; Thounaojam et al., 2011), spices (Padmanabhan & Prince, 2006) or pure compound from functional food (Rajadurai & Prince, 2007) are associated with reducing the risk of experimentally induced myocardial infarction. Wherein, improvement in plasma enzymatic and non-enzymatic antioxidants, TBARS and histopathology of cardiac tissue with regulated levels of HDL and LDL were observed. Also, combinatorial use of traditional Chinese herb (Cortex Moutan and Radix Salviae) on isoproterenol induced myocardial infarction in rats reported the enhancement of antioxidant defense system through activating of Nrf2 signaling and prevented apoptosis by regulation of Bax, Bcl-2 and Caspase-3 activation (Li et al., 2012).

The combinations of nutrient play synergistic role in maintaining the healthy lifespan of an individual. Studies with different dietary patterns had evident that good combination of diet will help to prevent the onset or progression of several chronic risk factors of CVDs (Alissa & Ferns, 2012; Fleming et al., 2013). Over the last 200 years, the “Western” diet has changed from being high in fiber, complex carbohydrates and lean meats to being high in, added sugars, solid fats, fatty meats, refined grains (Alissa & Ferns, 2012). This dietary shift contributes to the development of age-related chronic disease. Lack of knowledge about healthy diet, continues to be a huge gap between current eating patterns of the public versus those that are recommended. Even minor shifts from the current diet to ones recommended will provide additional cardioprotective benefits when used in combination with drug therapy.

Table 1.1: List of different pattern of diets successful against CVDs (Alissa & Ferns, 2012; Fleming et al., 2013)

	Traditional Okinawa	Mediterranean Diet	Dash Diet	Portfolio Diet	Ornish plan
Dietary Plan	yellow-orange root vegetables, green vegetables, legumes with moderate intake of fish/fish products and low intake of meat and dairy products.	Vegetables, fruits, nuts, whole grains, legumes, fish, monounsaturated fatty acids, moderate intake of alcohol; and low intake of red, saturated fatty acids, and processed meat	Fruits, vegetables and use of low-fat dairy products; preferential use of whole grains, fish, poultry and nuts, with reduced intakes of sweets, sugar-sweetened beverages, sodium and red meat	Plant sterols, viscous fibers primarily from oat, barley, soy protein and psyllium, and almonds.	Beans, legumes, fruits, grains, and vegetables; all sources of fat are restricted including plant sources such as avocados, seeds, and oils. The additional lifestyle Interventions implemented were aerobic exercise, stress management training, smoking cessation, and group psychological support
Clinical observations	Significantly lowering CVD risk factors, colon, breast and prostate cancer.	significantly lowering CVD risk factors, diabetes, adiposity and obesity	significantly lowering CVD risk factors	Significantly lowering C-reactive protein and other CVD risk factors	Significantly reducing arterial stenosis, angina, and LDL-C.

1.3 ANTHOCYANIN AND ITS PROPERTIES

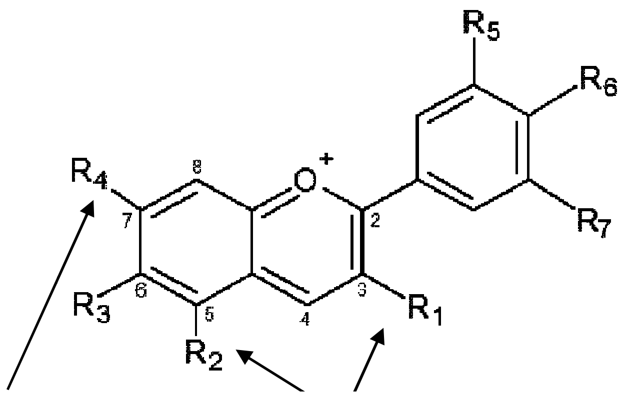
Anthocyanin (from the Greek *anthos*, a flower; and *kyanos*, dark blue) is well known as the natural pigment and is responsible for the blue, purple, violet, magenta, red and orange colors in flower, leaf, stem, fruit, seed, and the root of plants (Mazza & Miniati, 1993; Delgado et al., 2000). In plants anthocyanin play an important role in pollination and protection from environmental stress.

History

Since 1664 vegetable pigments are under investigation as mentioned in the second edition of "*The Anthocyanin pigments of plants*". In 1664, Robert Boyle had mentioned only about changes in red and green colored plant pigments in acid and alkaline solutions. Later on, in 1835 Marquat was first to coin the term anthocyanin with a belief that anthocyanin is dehydration product of chlorophyll. While, in 1862 Wigand identified that the oxidation of colorless tannin-like chromogen lead to the formation of anthocyanin. In 1849, Morot evidenced the presence of carbon, hydrogen and oxygen in the blue pigment from cornflower. To understand the chemical process involved in anthocyanin formation, Palladin in 1905, put forward a hypothesis that anthocyanin is formed from a flavone by the action of an oxidizing enzyme or oxidase. Also, Willstatter (1913) published a work on chemistry of anthocyanin wherein, it has been mentioned that all natural anthocyanins are present in the form of glucosides and its stability can be maintained by adding neutral salts or acid to the pigment solution. According to the Willstatter this phenomenon of anthocyanin is due to its quinonoid structure and tetravalent oxygen; which forms stable purple oxonium salt at neutral pH, red oxonium salt at acidic pH and blue oxonium salt at alkaline pH (Stafford, 1990).

Structure

Anthocyanins are consist of an aglycone or anthocyanidin bound to one or more sugar moieties at position-3 and in some, additional sugars are present at positions-5 or 7. The sugar moieties can be a mono or disaccharide wherein, the sugar molecules are commonly glucose, rhamnose, galactose or arabinose. Also, some anthocyanins are acylated with a phenolic or aliphatic acid attached to the sugar moieties. The commonly occurring anthocyanidins in foodstuffs are cyanidin (Cy), delphinidin (Dp), pelargonidin (Pg), peonidin (Pn), malvidin (Mv) and petunidin (Pt). Cyanidin, delphinidin and pelargonidin are non-methylated anthocyanidins and are widespread in nature compared to the three methylated anthocyanidins viz. peonidon, malvidin and petunidin.



- Binding site of Sugar moiety: monosides or biosides or diglucosodes
- One or more molecules of acyl acids (Sinapyl, p-coumaryl, ferulyl) are esterified to the sugar molecule

Name (Abbreviation)	R_1	R_2	R_3	R_4	R_5	R_6	R_7
Cyanidin (Cy)	OH	OH	H	OH	OH	OH	H
Delphinidin (Dp)	OH	OH	H	OH	OH	OH	OH
Pelargonidin (Pg)	OH	OH	H	OH	H	OH	H
Peonidin (Pn)	OH	OH	H	OH	OMe	OH	H
Petunidin (Pt)	OH	OH	H	OH	OMe	OH	OH
Malvidin (Mv)	OH	OH	H	OH	OMe	OH	OMe

Figure 1.3: Chemical structure of anthocyanin. Arrow represents the binding site of sugar moiety and acyl acids.

Sources

Natural source of anthocyanin includes variety of colored fruits, vegetables, spices and nuts. These includes berries, grapes, plums, peaches, pomegranate, red onion, red cabbage, egg plant, purple corn, purple sweet potato, black bean, pistachios and dates (Table 1.2) (Wu et al., 2006). The concentration and type of anthocyanin present in each fruit and vegetable vary considerably.

In the U.S., the average daily intake of anthocyanin has been estimated to be 215 mg during the summer and 180 mg during the winter (Kühnau, 1976). The common dietary sources include a variety of colored fruits and vegetables as well as fruit based processed foods and beverages like jelly, juices and red wine.

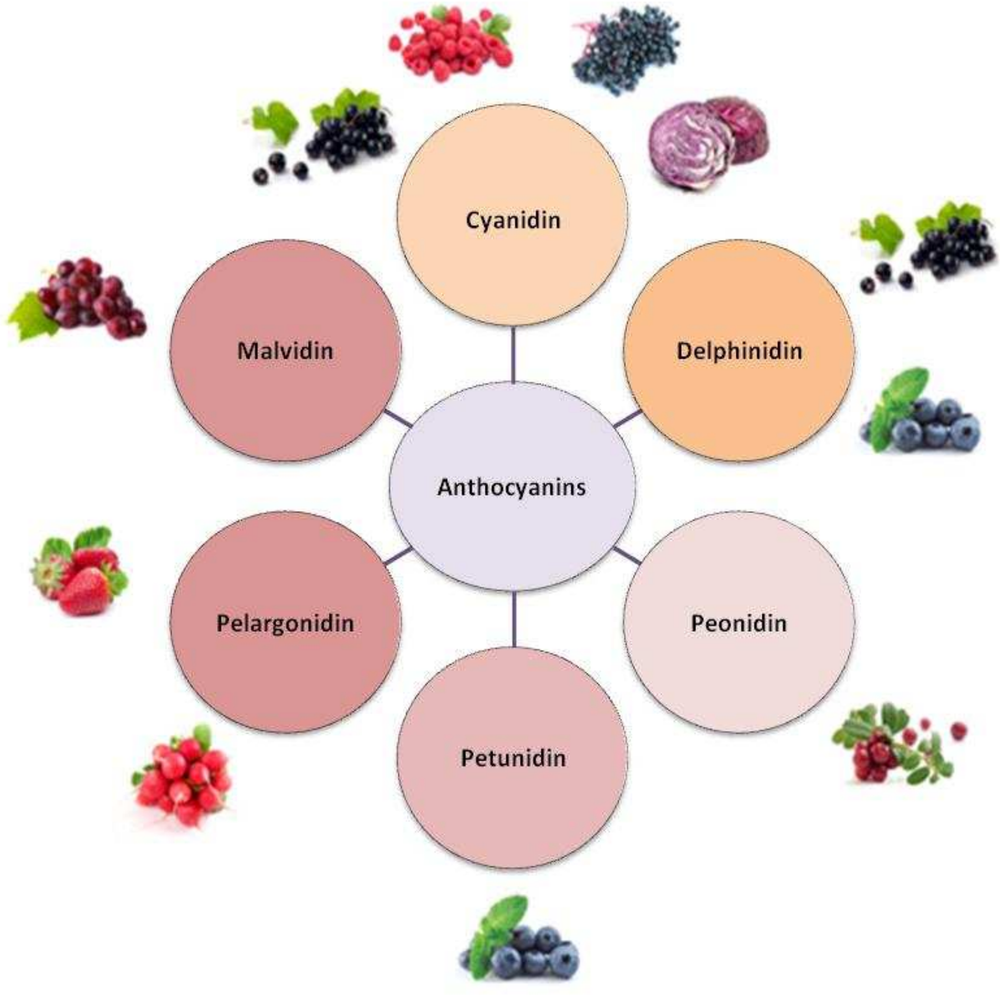


Figure 1.4: Different natural sources of anthocyanin

Table 1.2 List of different sources of anthocyanin. Red color represents vegetable or fruit enriched with specific group of anthocyanin

Anthocyanins with Different Sugar Moieties and Acylated or Nonacylated Groups in Common Foods						
mg/100 g (of fresh weight or form consumed)						
	Acylation		**Sugar acylation**			
Food	**Non-acylated ACN**	**acylated ACN**	**ACN-monoglycosides**	**ACN-diglycosides**	**ACN-triglycosides**	**Total ACN**
Fruits						
Blackberry	231 ± 56.4 (94%)	14.3 ± 13.9 (6%)	220 ± 89.1 (90%)	25.5 ± 28.8 (10%)	-	245 ± 68.0
Blueberry	348 ± 56.6 (96%)	16.4 ± 8.9 (4%)	365 ± 56.5 (100%)	-	-	365 ± 56.5
Sweet cherry	122 ± 21.3 (100%)	-	7.6 ± 3.0 (6%)	114 ± 20.9 (94%)	-	122 ± 21.3
Chokeberry	1480 (100%)	-	1480 (100%)	-	-	1480
Cranberry	140.0 ± 28.5 (100%)	-	140.0 ± 28.5 (100%)	-	-	140.0 ± 28.5 (100%)
Black currant	471 ± 115.7 (99%)	5.3 ± 6.7 (1%)	113 ± 33.1 (24%)	363 ± 83.8 (76%)	-	476 ± 115.4
Elderberry	1375 (100%)	-	742 (54%)	550 (40%)	82.6 (6%)	1375
Red Grape	20.8 ± 7.1 (78%)	5.8 ± 5.7 (22%)	26.7 ± 10.9 (100%)	-	-	26.7 ± 10.9
Concord grape	59.9 (50%)	60.2 (50%)	102 (85%)	18.5 (15%)	-	120
Black plum	124 ± 21.4 (99%)	0.5 ± 0.2 (1%)	96.7 ± 17.5 (78%)	27.8 ± 4.1 (22%)	-	124.5 ± 21.6
Black raspberry	687 (100%)	-	89.4 (13%)	440 (64%)	158 (23%)	687
Red raspberry	92.1 ± 19.7 (100%)	-	20.1 ± 9.3 (22%)	47.8 ± 9.4 (52%)	24.2 ± 15.8 (26%)	92.1 ± 19.7
Strawberry	21.0 ± 3.4 (99%)	0.2 ± 0.1 (1%)	19.6 ± 3.2 (93%)	1.6 ± 0.4 (8%)	-	21.1 ± 3.3
Vegetables						
Black bean	44.5 (100%)	-	36.1 (81%)	8.4 (19%)	-	44.5
Eggplant	85.7 (100%)	-	0.6 (1%)	71.5 (83%)	13.6 (16%)	85.7
Red cabbage	46.7 ± 7.3 (15%)	275 ± 33.8 (85%)	-	T	322 ± 40.8 (100%)	322 ± 40.8
Red leaf lettuce	2.1 ± 1.5 (95%)	0.1 ± 0.1 (5%)	2.2 ± 1.5 (100%)	-	-	2.2 ± 1.5
Red onion	11.0 (23%)	37.5 (77%)	33.6 (69%)	14.9 (31%)	-	48.5
Red Raddish	T	100.1 ± 30.0 (100%)	-	T	100.1 ± 30.0 (100%)	100.1 ± 30.0

Use as a Food Colorant

Since the ban of FD&C Red Nos. 2 and 4 and Red No. 40 (synthetic dyes), natural pigments has gained an interest among consumers. Pigments from natural sources are generally safe due to its health benefits and display a wide range of colors. Carotenoids from carrots, betalains from beets and anthocyanin from grapes, red cabbage or any other fruits and vegetables are some of the natural colors used in food industry (Sapers et al., 1981).

According to the numbering system used by the *Codex Alimentarius Commission*, anthocyanins are listed as natural colorant by the European Union (EU) legislation as product E163. In the U.S., the FDA (Food and Drug Administration) has a different list of natural colors that do not require certification, and anthocyanins can be obtained either from fruit or vegetable juices (Espín et al., 2000).

During 1879, enocyanin from grape skin was used as natural colorant in red wine. Slowly natural colorant from other source like elderberries, blueberries, cherry plums, black currant, red raspberries and red sweet potatoes gained an interest. A comparision between pigments from commercial black berry extract, enocyanin and red sweet potato recorded stable pigments from red sweet potato than commercial black berry extract and enocyanin (Imbert, 1966). Also, Espín et al., (2000) found anthocyanin based food extract (black chokeberry, black-thorn and strawberry) to have antioxidant capapcity, which was absent in commercial colorant ponceau 4R. Hence, this suggests the use of natural colors instead of synthetic colors as food colorant.

pH and chemical composition of anthocyanin

In aqueous solution anthocyanin exist in equilibrium between four species depending on pH namely, red flavylium cation, blue quinonoidal base, colorless carbinol pseudobase and the colorless chalcone (Brouillard, 1982; Brouillard & Dubois, 1977).

Below pH 2, anthocyanin is present in red flavylium cation form. Between pH 3-6, a rapid hydration of the flavylium ion at C-2 position leads to the formation of colorless carbinol pseudobase which can further get converted into chalcone pseudobase. At any pH, chalcone form is present in lower concentration than the carbinol form. Also, the reversion of chalcone to flavylium cation upon acidification is slower than reversion of carbinol pseudobase to flavylium cation (Fossen, Cabrita, & Andersen, 1998; Francis & Markakis, 1989). At slightly acidic to neutral pH deprotonation of the flavylium cation leads to the formation of quinonoidal bases. Further, between pH 6 and 7 deprotonation of the quinonoidal bases takes place with the formation of purplish resonance-stabilized quinonoid anions (Brouillard & Dubois, 1977).

Effect of temperature on anthocynain

Anthocyanin undergo thermal degradation, discoloration and decline in antioxidant activity at temperature higher than 70°C. Thermal degradation of anthocyanin may lead to the formation of two end products that is either aldehyde and benzoic acid or chalcone and coumaric acid. Earlier, opening of the pyrylium ring, loss of the B-ring, chalcone formation and formation of coumarin glucoside derivatives was considered as the degradation step of anthocyanin (Jackman & Smith, 1996; Markakis & Jurd, 1974; Markaris et al., 1957; Piffaut et al., 1994; Tanchev & Ioncheva, 1976). Later, Tanchev & Ioncheva, (1976) isolated seven compounds following thermal degradation of anthocyanins at 98°C, among which quercetin, phloroglucinaldehyde and protocatechuic acid were identified. In case of acylated anthocyanins, acyl-glycoside moieties were first split off from the flavylium backbone and finally ended up with phenolic acids and phloroglucinaldehyde (Sadilova et al., 2007).

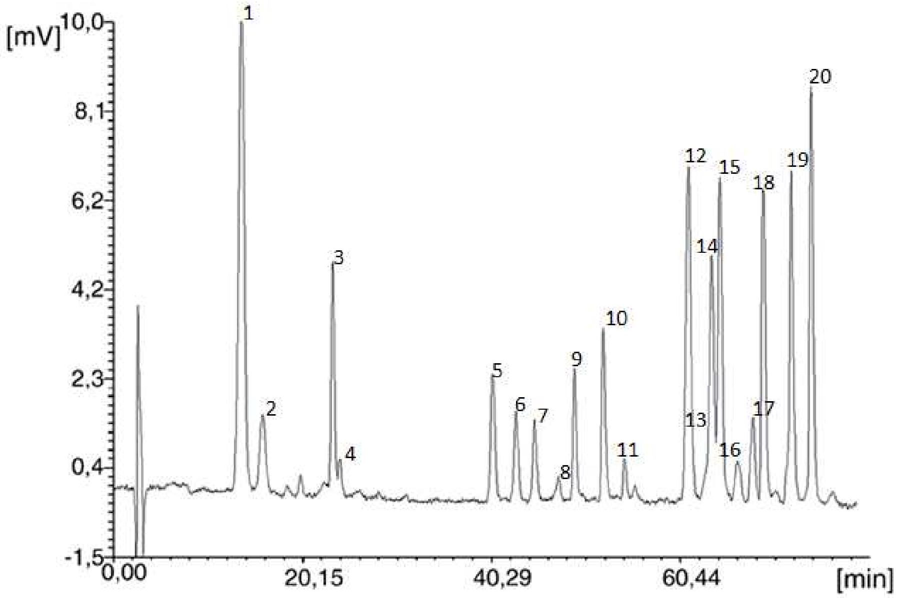
Light induced deterioration of anthocyanin

Light is also a significant factor in accelerating anthocyanin degradation. Fluorescent light can initiate anthocyanin degradation even at lower temperature (40°C). The degradation of anthocyanin by light follows first order kinetics (Attoe & Elbe, 1981). Photodegradation of anthocyanin proceeds with the formation of flavylium cation *via* carbinol pseudobase to the chalcone (Maccarone et al., 1985), also according to Furtado et al. (1993) direct photodegradation of flavylium cation can occur. Acylated anthocyanins are less susceptible to degradation by light (Giusti & Wrolstad, 2003).

Other factors like oxygen, hydrogen peroxide, ascorbic acid, and enzymes like polyphenoloxidase and peroxidase are found to play the role in anthocyanin degradation. Anthocyanin with degraded products of sugar like furfural and 5-hydroxymethylfurfural greatly are also more susceptible to pigment destruction (Daravingas & Cain, 1968).

1.4 HEALTH BENEFITS OF RED CABBAGE

Red cabbage (RC) (*Brassica oleracea* var. capitata f. rubra; family Brassicaceae, order - Brassicales), is herbaceous, biennial, dicotyledonous flowering plant. Red cabbage is native to southern Europe, but nowadays it is grown all over Europe. This variety is plentiful year round, but tastes the best when grown in cooler climates. RC is one of those veggie super foods, presenting more vitamin C than an orange (per serving), providing 61% of the daily vitamin K needs and healthy amounts of vitamin B5 (panthothenic acid), vitamin B6 (pyridoxine), and vitamin B1 (thiamin). RC is a fantastic source of antioxidant, phytochemical and contains a plethora of minerals, including potassium, manganese, iron, and magnesium. Among phytochemicals RC was found to be enriched with anthocyanin specifically cyanidin and delphinidin based, as found in our study and by other groups. Anthocyanins are having strong antioxidizing power of 150 flavonoids (Arapitsas et al., 2008; Arapitsas & Turner, 2008; Shama et al., 2012).



(Wiczkowski et al., 2013) - HPLC chromatogram of anthocyanin profile of red cabbage detected at 520 nm: cyanidin-3-diglucoside-5-glucoside (1), cyanidin-3-glucoside-5-glucoside (2), cyanidin-3-(sinapoyl)-triglucoside-5-glucoside (3), cyanidin-3-(sinapoyl)-diglucoside-5-glucoside (4), cyanidin-3-(caffeoyl)(p-coumaroyl)-diglucoside-5-glucoside (5), cyanidin-3-(feruloyl)- triglucoside-5-glucoside (6), cyanidin-3-(sinapoyl)-triglucoside-5-glucoside (7), cyanidin- 3-(feruloyl)(feruloyl)-triglucoside-5-glucoside (8), cyanidin-3-(feruloyl)-diglucoside-5- glucoside (9), cyanidin-3-(feruloyl)(sinapoyl)-triglucoside-5-glucoside (10), cyanidin-3- (caffeoyl)-diglucoside-5-glucoside (11), cyanidin-3-(p-coumaroyl)-diglucoside-5-glucoside (12), cyanidin-3-(caffeoyl)(p-coumaroyl)-diglucoside-5-glucoside (13), cyanidin-3- (feruloyl)-diglucoside-5-glucoside (14), cyanidin-3-(sinapoyl)-diglucoside-5-glucoside (15), cyanidin-3-(feruloyl)-glucoside-5-glucoside (16), cyanidin-3-(sinapoyl)-glucoside- 5-glucoside (17), cyanidin-3-(feruloyl)(feruloyl)-diglucoside-5-glucoside (18), cyanidin-3-(feruloyl)(sinapoyl)-diglucoside-5-glucoside (19), and cyanidin-3-(sinapoyl)(sinapoyl)-diglucoside-5-glucoside (20).

Figure 1.5: HPLC chromatogram profile of RC anthocyanin.

Phytochemical study

Harborne (1964) recorded that acylated groups are attached to sugar moieties (sophorosides) and Tanchev & Timberlake (1969) confirmed the presence of sinapic acid group in red cabbage anthocyanins using paper chromatography and thin layer chromatography. Phytochemical screening of water RC extract (powder and juice) recorded the presence of all phytochemicals (alkaloid, glycoside, steroid, flavonoid, saponin, tannin, terpenoid and phytosterol) while some of these are absent in methanolic and petroleum ether RC extract (Chauhan et al., 2016; Cruz et al., 2016).

Among the three cultivar of cabbage RC showed presence of more phenolics, vitamin C and leutine compare to white and savoy cabbage. Also, HPLC analysis of red cabbage cultivars viz. Koda, Haco POL and Kissendrup SWE recorded nine anthocyanins with different concentration and stability in all three cultivars. Haco POL cultivar showed strongest antioxidant power and more stability among other three cultivars (Pliszka et al., 2009).

Table 1.3: Antioxidant potential of different variety of cabbage

Vegetable	Cultivar	$ABTS^{•+}$	$DPPH^{•}$	$O_2^{•-}$
Red cabbage	Kissendrup	12.64±0.21	9.19±0.74	2.80±0.02
	Koda	9.81±0.74	6.76±0.46	2.73±0.09
Brussels sprouts	Ajax	7.04±.015	5.98±0.21	2.60±0.05
	Filemon	5.85±0.24	3.90±0.23	2.31±0.14
White cabbage	Almanag	1.81±0.12	1.00±0.04	4.35±0.21
	Tikana	1.46±0.03	0.77±0.05	10.07±0.92
	Vestri	1.34±0.07	0.90±0.07	7.40±0.35
Savoy cabbage	Langedijker	3.74±0.10	1.68±0.02	6.25±0.21
	60F/100	2.89±0.06	1.38±0.02	5.55±0.11

The values are expressed as mean ± SD, n≥3. **(Podsędek et al., 2014)**

HPLC analysis of RC showed cyanidin-3-diglucoside-5-glucoside and cyanidin-3-glucoside derivatives with various acylated (sinapic acid, coumaric acid and ferulic acid) groups connected to the glucoside or diglucoside as major anthocyanins (Figure 1.5). Also, physico-chemical evaluation of RC showed the presence of polar compounds (Shama et al., 2012) and have good antioxidant potential attributed to the presence of diacylated cyanidin then monoacylated cyanidin (Wiczkowski et al., 2013). RC anthocyanins showed high ε and λ_{max} in methanol than buffered solution at pH 1 while between pH 3-6 ε decreases and again increases at pH

7-8. Further, monoacylated and diacylated anthocyanins recorded different physico-chemical characteristics. Wherein, monoacylated ancthocyanins had more tendency to undergo degradation compare to diacylated anthocyanins as pH increases from 3-8, while at pH 1 minimal degradation was observed. Also, monoacylated anthocyanins showed different hue then diacylated anthocyanins at different pH range (pH: 1-8). Thus, these observations throw a light on nutritive value of RC, its health promoting factor and use as a food colorant. RC anthocyanins are found to be thermostable at <80ºC wherein, blanching and boiling resulted into loss of nutritional potential of RC while steaming showed no significant changes (Volden et al., 2008). Also, RC anthocyanin showed less photodegradation quantum at pH-7 and are colored at wide range of pH (pH-3 Pink; pH-5 Violet; pH-7 Blue) than anthocyanin from grape skin, black current and elder berry, thus can be a natural alternative to synthetic hue and relatively cheap source of pigment (Bridle & Timberlake, 1997; Giusti & Wrolstad, 2003; McDougall et al., 2007). According to the Piccaglia and team, RC is more preferable then berries due to its higher yield, more stable acylated anthocyanins and color. Also fertilizers have little or no effect on shaping the composition of anthocyanin (Piccaglia et al., 2002).

Bioavailibility of anthocyanin from RC

Not all nutrients taken by us are readily absorbed or utilized by our body in same form; hence bioavailability study will provide risk, safety and metabolic fates of nutrients. Structure of anthocyanin plays an important role in bioavailability and further intracellular activity. The number and organization of phenyl groups, availability of electron donating, electron withdrawing groups and positive charge on ring are responsible for preventing the formation of intracellular highly reactive free radicals and further oxidative stress. Yi et al. (2006) had observed that anthocyanin with more hydroxyl groups and less OCH_3 group have less bioavailability. Also, anthocyanin with glucose moiety showed more transport efficiency then anthocyanin with galactose moiety, thus sugar moiety attached to anthocyanin play a mechanistic role in transportation across the cell and regulation of intracellular homeostasis.

HPLC analysis of plasma and urine; *in vitro* gastrointestinal digestion and incubation with human microflora gave an information regarding bioavailability of anthocyanin rich RC extract. Bioavailability reports on human subjected to RC anthocyanin recorded 3 nonacetylated, 8 acetylated, 4 glucourinated and methylated anthocyanin in urine samples after 24 h consumption of RC (Charron et al., 2007; Kolodziejczyk et al., 2011; McDougall et al., 2007). Similarly, Wiczkowski et al. (2016) also studied the bioavailability of anthocyanin from fresh RC and fermented RC on human subjects. This comparison recorded the initial easy absorption of anthocyanin from fermented RC then fresh RC due to soft matrix of fermented RC. However, C_{max} of fresh RC anthocyanin (86.39 ± 3.51) was more than anthocyanin from fermented RC (64.02 ± 4.87) in plasma. DPPH assay and trolox assay of plasma recorded the highest antioxidant power at 2 h after consumption of RC. Where, anthocyanins from fresh RC showed more antioxidant power than fermented RC. Thus, both studies clearly depicts that some anthocyanins are absorbed in native form and some are converted into metabolites (methylated, glucourinated and sufated). Interestingly, i*n vitro* gastrointestinal digestion assay showed increase in total phenolic content during digestion with decrease in anthocyanin concentration and antioxidant capacity after pancreatic-bile digestion (Podsędek et al., 2014). Also, incubation with human fecal microflora caused further decline in anthocyanin content. Thus, intact anthocyanin in gastric and products of their decomposition in small and large intestine may be responsible for the antioxidant activity and other physiological effects after consumption of RC.

Toxicity evaluation

Toxicokinetic study provides information regarding risk and safety related to test compounds. Swiss albino mice administered with RC extract (1000 mg/kg and 2000 mg/kg body weight) for 28 days recorded no adverse effect on physiology and metabolic fates. While, mice fed with RC extract (3000 mg/kg body weight) showed decrease in food intake, body weight gain, RBC count and hemoglobin content. However, mice administered with single dose of RC extract (1000 mg/kg, 2000 mg/kg, 3000 mg/kg, 4000 mg/kg and 5000 mg/kg body weight) were live and no adverse behavioral changes were observed. Hence, it has been extrapolation from this data that consumption of 4041 gm RC by 65 kg human is safe and devoid of side effects as per NOAEL (Thounaojam et al., 2011).

Antibacterial and antifungal activity

30 KDa protein isolated from red cabbage seed showed antifungal and antibacterial. Protein inhibited mycelia growth in *Mycosphaerella arachidicola* (5 µM), *Setospaeria turcica*, *Bipolaris maydis* and *Candida albicans* growth (96 µM) at different concentrations. Also, 53 µM of isolated protein was potent against *Pseudomonas aeruginosa* only; among other gram positive and one gram negative bacteria (*Bacillus cereus, B. megaterium, B. subtilis, Proteus vulgaris, and Mycobacterium phlei respectively*) (Ye et al., 2011).

Antioxidant and free radical scavenging activity

Anthocyanin is well known for its antioxidant property and this counterpart has been well studied also with anthocyanin from RC. Several studies had been proposed taking this into consideration in order to seek an answer the mechanistic approach of RC anthocyanins against pathological diseases. These studies evidenced that beside its antioxidant property it can also increase the intracellular antioxidants and regulate the intracellular homeostasis. A study with ROS induced hyperthyroidism had evidenced that anthocyanin rich RC extract is potent in regulating the hyperthyroidism condition by increasing the levels of intracellular antioxidants capacity, thyroid stimulating hormone and reducing the level of thyroxine, triiodothyronine and malonaldehyde in serum (Majeed & Al-Azzawie, 2012). Similarly, a study with paraquat which is known for the production of highly reactive superoxides and hydrogen peroxide through intracellular redox cycling reaction, evidenced the protective role of acylated anthocyanin from RC against paraquat induced liver pathogenesis and cholesterol metabolism. Wherein, increase in liver mitochondrial antioxidants (superoxide dismutase and catalase) with improved food uptake, serum cholesterol level, lung and liver weight were recorded (Igarashi et al., 2000).

Cardio protective potential

Increase in death burden due to cardiovascular diseases (CVDs) tends the research fraternity towards finding more healthy way to improve the condition instead of harmful side effects of CVD drugs. Several studies have been performed with natural compounds and factors causing CVDs to open up new way to treat or prevent and reduce the burden of deaths due to CVDs. Anthocyanin rich red cabbage extract

(ARCE) showed cardioprotection against H_2O_2 and High fat diet induced damage. Wherein, ARCE was found potent to reduce oxidative stress in H9c2 cells (Devkar et al., 2012). Also, high fat diet/atherogenic diet mediated reduction in intracellular antioxidant status, increase in lipid peroxidation and further cardiac damage was improved by supplementation of ARCE (100 mg/kg body weight) along with atherogenic diet (Sankhari et al., 2012).

Nephro protective activity and Anti-diabetic activity

Nephropathy is condition usually found in case of diabetic patients, due to overproduction of ROS. A study hypothesized based on the antioxidative property of anthocyanin evidenced that RC extract not only regulated hyperglycemic condition but also prevented nephropathy. Wherein, daily oral ingestion of 1 g/Kg of RC extract for 60 days reduced blood glucose level and loss in weight gain of streptozotocin (STZ)-diabetic rats. Whereas, untreated STZ-diabetic rats showed increase in weight loss and blood glucose level. Also, increase in kidney weight; increase in urea and creatinine content in serum and decrease in renal antioxidant status was restored by giving pretreatment of RC extract to STZ-diabetic rats (Kataya & Hamza, 2008).

Anti-inflammatory and Analgesic Activity

Different bioactive components of red cabbage showed differential immune-modulatory properties. Lin & Li (2010) had shown in their previous investigation that extract rich in phenolics and flavonoids increases IL10 (anti-inflammatory cytokine) and decreases IL6 (pro-inflammatory cytokine), while the protein rich red cabbage extract resulted increase in IL20 (anti-inflammatory cytokine) and decrease in TNF-α with no significant change in LPS induced IL6 secretion. Also, ethanolic extract of RC showed improvement in dextran sulfate sodium (DSS) and trinitrobenzene sulfonic acid (TNBS) model of inflammatory disorder in gastrointestinal tract (Zielińska et al., 2015). Wherein, ethanolic RC extract was potent in improving the acute TNBS model of colitis completely by reducing inflammatory markers (IL-1β and TNF-α), immune cell infiltration, ulcer score, MPO activity, colon length and colon thickness; while in chronic TNBS model ethanolic RC extract was not potent to improve the colon thickness; and in DSS model it only improved the ulcer score, immune cell infiltration and myeloperoxidase activity. Hence, this study represents the potent anti-inflammatory activity not antioxidant activity of ethanolic RC extract in improving the condition of inflammatory disease mouse model.

Red cabbage showed more inhibitory activity against COX-1 and less inhibitory activity against COX-2 than purple carrot (rich in monoacylated anthocyanins) and standard cyanidin 3-*O*-coumaroyl-sambubioside-5-*O*glucoside. Wherein, the inhibitory activity against COX-2 is due to the anti-inflammatory or antitumoregenic effect of an extract and specifically attributed to monoacylated anthocyanins as purple carrot is rich in monoacylated anthocyanins (Mizgier et al., 2016). Also, Mizgier et al. (2016) have mentioned about synergistic or antagonistic approach of anthocyanin and phenolic acid found in extract and standard anthocyanin used by them showed less inhibitory activity then extract.

Anticancer activity

2-amino-1-methyl-6-phenylimidazo (4,5-b) pyridine (PHiP) is carcinogenic agent usually found in cooked food like meat and chicken; PHiP formation depends on duration and temperature of cooking. 1,2-Dimethyl hydrazine (DMH), is a well known carcinogenic agent to induce colon cancer, when given with PHiP influenced the carcinogenesis. After giving DMH to rats followed by RC color with PHiP showed preventive effect of RC color against the carcinogenesis with Decrease in hyperplasia and adenocarcinoma. Also, Hagiwara and team recorded that RC color reduced the formation of aberrant crypt foci in rat colon induced by PHiP alone. Hence, RC color was potent in reducing carcinogenesis induced by alone PHiP or by combination of DMH and PHiP (Hagiwara et al., 2002).

Chemo protective activity

Later on, a study with RC extract and Irinotecan (CPT-11) evidenced that anthocyanin rich RC extract can provide health benefits against mucositis (a loss of mucus producing goblet cells) which is main side effect found during chemotheraphy of colorectal cancer. CPT-11 is widely used to treat lung, gastric, cervical, ovarian cancer and recommended as first and second line therapy for colon cancer. In addition to anticancer activity, CPT-11 has high prevalence of gut toxicity with less effective therapeutic available against it. Anthocyanin rich RC extract (50 and 100 mg/kg for 7 days) not only prevented the loss of goblet cells and diarrhea; also reversed the adverse effect of CPT-11 on autophagosomes in dose dependent manner. Wherein, anthocyanin rich RC extract also preserved height of villi, epithelial cell surfaces, intestinal tight junction (ZO-1) and transepithelial electric resistance in small intestine, colon and Caco2 cells more efficiently than loperamide which is well known medicine to cure only symptoms not the cause of diarrhea (Tong et al., 2017).

RC extract or RC seed protein showed anticancer activity on cervix adenocarcinoma (HeLa), hepatocellular carcinoma (HepG2) and nasopharyngeal cancer (NE-1) (Hafidh et al., 2013; Ye et al., 2011). RC extract induced cytotoxicity in HeLa and HepG2 cells (23.38 and 28.66 mg/ml respectively) at very low concentration compare to human peripheral blood mononuclear cells (normal cells;

251.28 mg/ml), while protein from RC seed suppressed the proliferation of NE-1 and HepG2 at 50 and 90 µM after 48 h. RC extract induced TNFα expression in a dose dependent manner, also increased apoptotic index in a dose dependent manner and time dependent manner at particular dose with maximum apoptosis at 48h. However, cell cycle analysis showed that RC treatment in cancer cells induces apoptosis by increasing the cyclin-cdk inhibitors (P27 at 12 h; P21 and P53 at 16 h) and decreasing the levels of cyclin D and E only after 16h with cell cycle arrest at 16 h. Also increase in bax/bcl-2 ratio (16 h), caspase 9 (12 h) and caspase 7 (16 h in HeLa; 12 h in HepG2) shows the differential anticancer activity of RC extract may be due to presence of other phenolic components in crude extract.

Since long time, sulforaphane is a known for its anticancer activity. Sulforaphane is present in crucifer vegetables (broccoli, brussels sprout, green cabbage, red cabbage, Chinese kale and turnip), among these vegetables sulfarophane was found in less concentration from red cabbage and kale. Thus, red cabbage recorded only 27% of cytotoxicity against A549 cell (Farag & Motaal, 2010). While sulfarophane extracted from red cabbage showed anticancer activity against human epithelial carcinoma cell line HEp-2 at 58.96 µg/ml concentration and crude RC extract showed anticancer activity at 113 µg/ml (Devi & Thangam, 2012). Hence, this records the importance of sulfarophane present in red cabbage as an anticancer agent.

1.5 RATIONALE OF THE STUDY

There are series of studies showing protective potential of RC extract against several risk factors. Also, Protein from RC seed was found to have antibacterial and antifungal activity as described in this chapter. In addition, potential of hydromethanolic RC extract against hypercholesteriomia was observed by our team. After the discovery of SOD in 1969, exponential growth in free radical research has been observed. Reports over last several years have shown the role of oxidative stress in progression of CVDs, as diverse as heart failure, ischemia reperfusion, endothelial dysfunction, hypertension, and atherosclerosis. Treatment with antioxidants or with natural source of antioxidant or with superoxide dismutase has been shown to be effective in reducing markers of oxidative stress and improving functional parameters such as intracellular antioxidant status, relaxation of endothelium and prevention of LV remodeling. H_2O_2 and isoproterenol (ISO) induced oxidative stress model is widely used to study the role of free radicals in pathogenesis of CVDs. Hence, this study was designed to determine the role of anthocyanin rich red cabbage extract (ARCE) pretreatment in improving intracellular homeostasis and preventing cellular damage in presence of oxidative stressors.

1.6 HYPOTHESIS OF THE STUDY

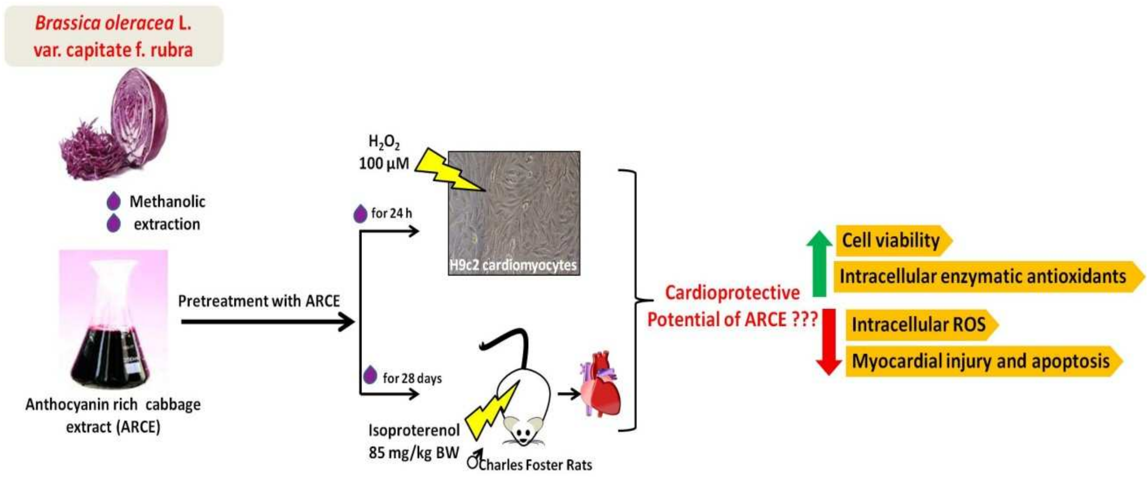
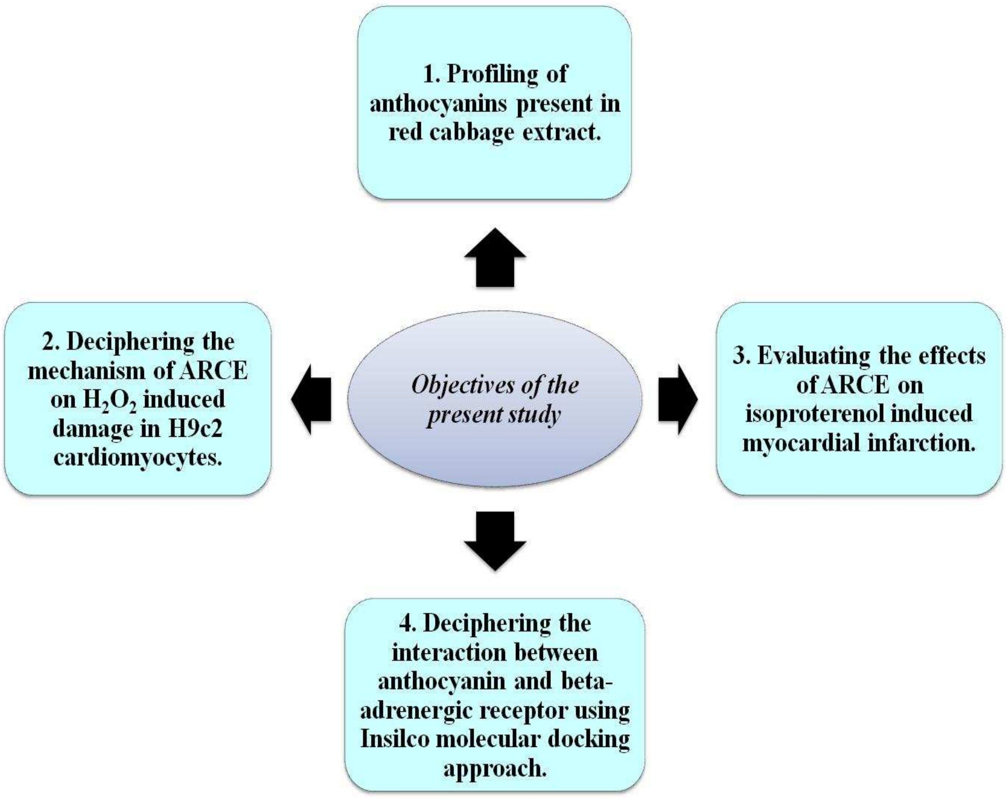


Figure 1.6: Schematic representation of proposed hypothesis. Present study was focused on the role of ARCE pretreatment in modulating the intracellular homeostasis of cardiomyocytes and preventing cellular damage in presence of oxidative stressors.

Based upon the proposed hypothesis following are the objectives for this study:



1.7 SIGNIFICANCE OF THIS STUDY

This study will provide an impact on role of dietary flavonoids in regulating the intracellular homeostasis and protecting cells from the forthcoming free radical stressors. This study will be helpful to pharma industry and food industry to involve anthocyanin for health benefits. In addition, elucidation of β1AR and anthocyanin interaction can be helpful to understand the mechanism of anthocyanin mediated prevention of myocardial infarction (MI).