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Bioefficacy of red cabbage against hypercholesterolemic diet mediated oxidative stress

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Abstract

Background: The shift towards hypercaloric diets and sedentariness has raised lifestyle related disorders and escalated health care cost. In order to tackle this epidemiological transition, healthy, affordable food choices should be introduced in the routine menu. In this context, red cabbage is known for its rich phytochemistry, minerals, antioxidant vitamins and dietary fiber. Considering these evidences, red cabbage leaves and its extract were assessed against hypercholesterolemia and associated oxidative stress.

Methods: In bioefficacy assessment trial (12 weeks), there were two dietary regimens; normal and hypercholesterolemic (1% cholesterol) that were further split into three groups each. In both feeding trials, red cabbage leaves (20%) and its extract in dose equivalent to red cabbage leaves were assessed against control diets. At termination of trial, serum lipidemic parameters and oxidative stress biomarkers were assessed to test the efficacy of diets.

Results: In hypercholesterolemic rabbits, red cabbage leaves showed significant reduction in cholesterol, LDL-c and triacylglycerol levels i.e. 15.19, 18.09 and 9.42% than extract administered groups; 10.79, 12.24 and 5.72, respectively. Besides, red cabbage leaves also portrayed momentous enhancement of superoxide dismutase (SOD) and catalase (CAT) activity up to 13.29 & 17.63% by lowering lipid peroxidation by 27.86% in hypercholesterolemic diet fed groups, whereas red cabbage extract administered group depicted relatively lesser amelioration in lipid peroxidation i.e. 21.42%.

Conclusions: Red cabbage leaves possess higher ameliorative potential against altered lipidemic profile and lipid peroxidation as compared to its extract thus explains its ability to prevent exhaustion of endogenous antioxidant enzymes; SOD and CAT.

Keywords: Red cabbage leaves, Red cabbage extract, Lipid profile, Superoxide dismutase, Catalase, Lipid peroxidation

Background

The nutritional transition towards junk foods has become the foremost reason for dramatic prevalence of obesity, coronary heart disease (CHD) and cancer [1–3]. The consumption of junk food or hypercaloric foods increases lipid load in cells of the body or may lead to augmented generation of Reactive Oxygen Species/Metabolites (ROS/ROM) i.e. liable to attack on biological membranes resulting in redox mediated lipid peroxidation, organ damage and depletion of the activity of endogenous antioxidants enzymes like superoxide dismutase (SOD) and catalase (CAT). This situation where free radicals increases over antioxidants within the physiological system is termed as oxidative stress [4–6]. In contrary, exogenous antioxidants support endogenous enzymatic antioxidants like SOD and CAT that are involved in the detoxification of free radicals (superoxide anion & hydrogen peroxide) and lipid peroxides to non-toxic metabolites [6–8].

In this regard, there is a rising interest in plant extracts or biologically active ingredients to alleviate such dysfunctions [7, 9]. The healthy dietary interventions provide sufficient amounts of exogenous antioxidants that strengthen endogenous antioxidants ultimately improve

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natural defense against numerous ailments [10, 11]. Large population survey in China has inversely associated the sufficient intakes of vegetables and legumes with diseases [2, 6, 12]. Amongst various vegetables, brassica vegetables such as broccoli, cabbage, cauliflower and brussels sprouts contribute positively towards health perspectives [10, 13, 14]. Recently, phenolic compounds of cabbage have gained immense attention to limit LDL oxidation i.e. a major determinant of atherosclerotic events and possess higher reducing power as compared to vitamin C [6].

Cabbage (*Brassica oleracea* L.) is an important crop that belongs to family Brassicaceae or Cruciferae. It carries abundant proportions of fiber, vitamins, minerals and health boosting compounds [15]. The most familiar varieties of cabbage include green, red, chinese and savoy [9]. The antioxidant capacity of cabbage heads is strongly influenced by genome, geographical locations and environment [11]. Commonly, cruciferous vegetables are consumed as salad however, in Arabic folkloric practices, cabbage juice is considered as an effective approach to protect against lifestyle related disorders [3]. Among cruciferous vegetables, red cabbage is well-known for its pigmented compounds named as anthocyanins i.e. quantified in abundance to that of black carrot, blackcurrant, grape skin and elderberry. Anthocyanins are potent antioxidants that serve as an attractive alternative in comparison to synthetic dyes, minimizing public safety concerns [15, 16]. Besides anthocyanins, cabbage also possesses some other hypolipidemic components including flavonoids, ascorbic acid and isothiocyanates along with other health protective moieties exist like hydroxycinnamic residues, β-carotenes, lutein, zeaxanthin, etc. [2, 3, 9]. Considering the aforesaid facts, the interest of this study was to explore the potential of whole red cabbage plant matrix and its extract against hypercholesterolemia and related oxidative stress in rabbits.

Methods

Chemicals and preparation of raw materials

In the current study, red cabbage (variety: Red Globe), Botanical name: *Brassica oleracea var. Capitata* was procured Ayub Agriculture Research Institute (AARI), Faisalabad, Pakistan. The cabbage samples were randomly selected during winter season, based on their quality attributes and washed to remove foreign matters & other impurities followed by refrigeration. The selected cabbage leaves and its extract were tested for their efficacy against hypercholesterolemic (high cholesterol) diet induced oxidative stress in male white New Zealand rabbits. For extract preparation, red cabbage leaves were subjected to an electric blender followed by filtration of juice extracted using muslin cloth to remove fiber portion according to the protocol of Al-Dosari [3]. For bioassays, diagnostic kits were purchased from Sigma-Aldrich Bioassay (Bioassays Chemical Co. Germany) and Cayman Chemicals (Cayman Europe, Estonia).

Experimental paradigms

Sixty male white New Zealand rabbits were acquired from National Institute of Health (NIH), Islamabad, Pakistan and housed in the Animal Room of the National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan. Purposely, rabbits were acclimatized to environmentally controlled room with temperature of 23 ± 2 °C and relative humidity of 55 ± 5%. Rabbits were fed on normal diet i.e. green leafy vegetables (composition on dry matter basis: 10.43% crude protein, 23.95% crude fiber, 9.85% ash content, 3.96% crude fat and 51.81% carbohydrate) and provided with tap water ad libitum for one-week prior experimentation (NIH Publications No. 8023, revised 1978). The experimental procedures were carried out between 9 a.m. to 11 a.m. During twelve-weeks study, rabbits were divided into six groups based on diet fed, each group comprises of ten rabbits. In "N" group, rabbits were fed with normal diet i.e. green leafy vegetables free from any treatment, whereas 1% cholesterol in 2% corn oil was daily administered to "H" group rabbits using a gastric tube along with green leafy vegetables, named as hypercholesterolemic diet. In "NRC" group, rabbits were supplemented with red cabbage leaves (20% of their diet) plus normal diet. Whilst, "NRCE" group was administered with red cabbage extract (extract yield; 15 mL/100 g F.W.) in equivalent dose to cabbage leaves fed to NRC group, along with normal diet. Likewise, "HRC" and "HRCE" groups were provided with 20% red cabbage leaves and its equivalent extract in their respective groups in addition to hypercholesterolemic diet (Table 1). At termination of trial, sera were collected in non-coated tubes (yellow capped vials) to assess serum lipidemic profile and related oxidative stress biomarkers using Microlab-300, Merck, Germany.

Table 1 Animal study plan

Diets	Experimental groups	Description
Normal diet	N	Normal diet
	NRC	Normal diet + red cabbage
	NRCE	Normal diet + red cabbage extract
Hypercholesterolemic diet	H	Hypercholesterolemic diet
	HRC	Hypercholesterolemic diet + red cabbage
	HRCE	Hypercholesterolemic diet + red cabbage extract

Lipidemic aspects

The serum lipid profile; high density lipoprotein (HDL) was determined by HDL precipitant method using commercially available Ecolin kits (Merck, Germany), whereas total cholesterol (TC) and triacylglycerol (TAG) were measured using commercial kits; Fluitest Chol (Cholesterin CHOD-PAP) and Fluitest TG (Triglyceride GPO-PAP) kits (Biocon, Vohl-Marienhagen, Germany), respectively. The analyses were performed using semi-automated clinical chemistry analyzer; Microlab 300, Merck, Netherland. Furthermore, non-HDL, LDL and VLDL were calculated using Friedewald formula. The lipidemic ratio including Atherogenic Index (AI), Cardiac Risk Ratios (CRRs), HDL to total cholesterol ratio (HTR) and Anti-atherogenic Index (AAI) were also calculated [17–21].

Low density lipoprotein-cholesterol (LDL-c)

$$\text{LDL concentration} = \text{Total cholesterol} - \text{HDL} - \text{VLDL}$$

Very low density lipoprotein-cholesterol (VLDL-c)

$$\text{VLDL concentration} = \frac{\text{Triglycerides}}{5}$$

Non-high density lipoprotein-cholesterol (non-HDL-c)

$$\text{nHDL (LDL + IDL + VLDL)} = \text{Total cholesterol} - \text{HDL}$$

Atherogenic index (AI)

$$\text{Atherogenic index} = \frac{\text{nHDL}}{\text{HDL}}$$

Cardiac risk ratio (CRR)

$$\text{Cardiac risk factor ratios} = \frac{\text{LDL}}{\text{HDL}}$$

HTR (HDL-cholesterol to total cholesterol ratio)

$$\text{HTR}\% = \frac{\text{HDL}}{\text{Total cholesterol}} \times 100$$

Anti-atherogenic index (AAI)

$$\text{Antiatherogenic index} = \frac{\text{HDL}}{\text{nHDL}}$$

Oxidative stress biomarkers

The collected serum from each animal was directly employed to endogenous antioxidant activity analyses such as superoxide dismutase (SOD) and catalase (CAT) activities while lipid peroxidation (MDA) in the serum samples was assessed by thiobarbituric acid reactive substances (TBARS) assay [22, 23].

Superoxide dismutase (SOD)

The superoxide anion, generated by xanthine-xanthine oxidase, reacts with NBT (yellow coloration), forming blue colored formazan i.e. measured at 560 nm. The percent inhibition of NBT reduction is a measure of SOD. For the determination, 50 μL of sample was mixed with 1 mL of 250 μL of PBS (pH 5), 100 μL of methionine, 100 μL of trition X, 50 μL of nitroblue tetrazolium (NBT) and 400 μL of distilled water. Afterwards, the reaction mixture was kept under UV light for 15 min. Then the resultant mixture (50 μL) was added in 96-well plate along with 50 μL of riboflavin and absorbance was measured against blank at 560 nm. One unit of SOD represents the amount of enzyme required to inhibit the rate of NBT oxidation by 50% at 25 °C. The enzyme activity in serum was calculated in Unit/mL.

Catalase (CAT)

The principle is based on decomposition rate of H_2O_2 by catalase at 240 nm. Purposely, 100 μL of sample was treated with 100 μL of 5.9 mM of H_2O_2 i.e. freshly prepared in phosphate buffer saline. The disappearance of H_2O_2 was monitored at 240 nm for 1 min at 25 °C. Catalase activity was calculated using extinction co-efficient of 0.0436 $mM^{-1}\,cm^{-1}$. The enzyme activity was calculated as Unit/mL in sera.

Lipid peroxidation (MDA)-TBARS assay

Malondialdehyde (MDA) is a dialdehyde of malonic acid i.e. present in the sample reacts with thiobarbituric acid (TBA) in the kit at high temperature under acidic conditions, forming MDA-TBA adduct with pink coloration, whose concentration was estimated using spectrophotometer. Purposely, 300 μL of MDA solution (20% TCA solution containing 0.5% thiobarbituric acid) was vortexed along with 50 μL of sample and heated on water bath at 50 °C for 50 min. Afterwards, the reaction mixture was cooled in iced water bath for 10 min followed by 20 min stay at room temperature. After stabilizing, each sample mixture was centrifuged at 10 °C, 3000 rpm for 15 min. The resultant supernatant i.e. an organic layer was placed in a 96-well plate and absorbance was recorded at 532 and 600 nm. The similar procedure was employed for blank & standard and final values were measured using standard curve.

Statistical analysis

The obtained data of the current study were subjected to statistical modeling to test the efficiency of each

parameter. Purposely, handling and summarizing of data was accomplished via Microsoft Excel (version 2013). Further, the statistical software Statistix 8.1 was employed as a statistical tool in which one-way ANOVA under Completely Randomized Design (CRD) was applied to assess the level of significance ($p < 0.05$ & $p < 0.01$) followed by comparison of means through Tukey's honest significant difference (HSD) test [24].

Results

Serum lipidemic profile

Cholesterol

The F values in Table 2 pertaining to cholesterol indicated significant effect of treatments in different groups of normal and hypercholesterolemic diet fed rabbits during the experimentation period. Means for normal rabbits depicted maximum cholesterol level as 73.02 ± 3.69 mg/dL in (N group) that significantly reduced to 70.09 ± 3.16 mg/dL (NRCE group) and 69.12 ± 3.56 mg/dL (NRC group). In hypercholesterolemic diet fed rabbits, the maximum decrement was viewed in HRC group as 124.09 ± 5.56 mg/dL followed by 130.52 ± 6.94 mg/dL in HRCE group as compared to control (146.31 ± 7.29 mg/dL). Thus, red cabbage was found more effective in lowering cholesterol as compared to its extract in both dietary patterns; normal and hypercholesterolemic. In normal rabbits, the dietary interventions based on red cabbage and its extract resulted in 5.34 and 4.01% decline in cholesterol profile, accordingly. In hypercholesterolemic rabbits, red cabbage and its extract depicted obvious reductions up to 15.19 and 10.79%, respectively.

Triacylglycerol

The statistical analysis showed that tested diets imparted non-significant impact on triacylglycerol in normal rabbits. Nonetheless, the impact of treatments was momentous in hypercholesterolemic diet mediated oxidative stressed rabbit. The mean value for triacylglycerol in normal control rabbits was 89.10 ± 4.61 mg/dL that decreased slightly to 85.06 ± 3.81 and 86.14 ± 4.45 mg/dL in NRC and NRCE groups, respectively. The triacylglycerol in hypercholesterolemic rabbits was reduced to 105.08 ± 5.22 mg/dL (HRC) and 109.37 ± 4.90 mg/dL (HRCE) as compared to positive control group (116.01 ± 5.20 mg/dL) as viewed in Table 2. Conclusively, red cabbage showed better tendency to suppress triacylglycerol i.e. up to 9.42% as compared to its extract 5.72%, in hypercholesterolemic rabbits, however the decrement was minor in normal study.

LDL-cholesterol

The F values indicated that dietary inclusion of red cabbage and its extract imparted significant impact on LDL-c in both normal and hypercholesterolemic dietary groups. In normal animals, reduction in LDL-c was 18.47 ± 1.41 mg/dL (NRC) and 18.92 ± 1.40 mg/dL (NRCE) from 20.09 ± 1.13 mg/dL (N) while maximum down-regulation was viewed in hypercholesterolemic diet fed groups from 92.99 ± 4.36 mg/dL (H) to 72.01 ± 3.87 mg/dL (HRC) & 77.16 ± 4.21 mg/dL (HRCE) as observed in Table 2. Thus, red cabbage reduced the LDL-c up to 8.07 & 18.09% in normal & hypercholesterolemic diet dependent rabbits, respectively whereas, red cabbage extract down-regulated the LDL-c by 6.46% in normal rabbits and 12.24% in hypercholesterolemic diet induced oxidative stressed rabbits.

VLDL-cholesterol

The statistical study related to VLDL-c (very low density lipoprotein-cholesterol) in normal rabbits differed non-significantly with respect to treatments, whereas for hypercholesterolemic rabbits, the VLDL-c in different

Table 2 Effect of diets on lipidemic profile of rabbits

Experimental groups	Parameters (mg/dL)					
	CHOL	TAG	LDL-c	VLDL-c	n-HDL-c	HDL-c
N	73.02 ± 3.69^{a}	89.10 ± 4.61	20.09 ± 1.13^{a}	17.82 ± 0.86	39.51 ± 2.73^{a}	33.51 ± 1.54
NRC	69.12 ± 3.56^{b}	85.06 ± 3.81	18.47 ± 1.41^{b}	17.01 ± 0.75	35.48 ± 2.32^{b}	33.64 ± 1.52
NRCE	70.09 ± 3.16^{ab}	86.14 ± 4.45	18.92 ± 1.40^{ab}	17.23 ± 0.97	36.02 ± 2.51^{b}	34.07 ± 1.92
F value	3.42^{*}	2.36^{NS}	4.01^{*}	2.33^{NS}	7.51^{**}	0.32^{NS}
H	146.31 ± 7.29^{a}	116.01 ± 5.20^{a}	92.99 ± 4.36^{a}	23.20 ± 1.52^{a}	116.19 ± 6.24^{a}	30.12 ± 1.32
HRC	124.09 ± 5.56^{b}	105.08 ± 5.22^{b}	72.01 ± 3.87^{c}	21.02 ± 1.68^{b}	93.03 ± 5.95^{b}	31.06 ± 1.73
HRCE	130.52 ± 6.94^{b}	109.37 ± 4.90^{b}	77.16 ± 4.21^{b}	21.87 ± 1.46^{ab}	99.03 ± 6.33^{b}	31.49 ± 1.26
F value	29.66^{**}	11.63^{**}	69.35^{**}	5.01^{*}	37.86^{**}	2.32^{NS}

Data values represent mean ± SD ($n = 10$); One-way ANOVA followed by Tukey's HSD multiple comparison tests; Means carrying different superscripted letters differ significantly

^{NS} Non significant ($p \geq 0.05$), *CHOL* Cholesterol, *TAG* Triacylglycerol, *LDL-c* Low density lipoprotein cholesterol, *VLDL-c* Very low density lipoprotein cholesterol, *n-HDL-c* Non-High density lipoprotein cholesterol, *HDL-c* High density lipoprotein cholesterol, *N* Normal diet, *NRC* Normal diet + red cabbage, *NRCE* Normal diet + red cabbage extract, *H* Hypercholesterolemic diet, *HRC* Hypercholesterolemic diet + red cabbage, *HRCE* Hypercholesterolemic diet + red cabbage extract

* = Significant ($p < 0.05$); ** = Highly significant ($p < 0.01$)

groups varied significantly as a function of treatments. In normal animals, the VLDL-c values were ranging from 17.01 ± 0.75 to 17.82 ± 0.86 mg/dL however, in hypercholesterolemic rabbits, the said parameter decreased from 23.20 ± 1.52 mg/dL (H) to 21.02 ± 1.68 mg/dL (HRC) and 21.87 ± 1.46 mg/dL (HRCE) as expressed in Table 2.

n-HDL-cholesterol

The F values relating to n-HDL-c (Table 2) presented significant variation in both normal and hypercholesterolemic animals with respect to diet fed. The means of n-HDL-c in normal rabbit groups; N, HRC and NRCE were 39.51 ± 2.73, 35.48 ± 2.32 and 36.02 ± 2.51 mg/dL, whereas the means for the said trait were 116.19 ± 6.24, 93.03 ± 5.95 and 99.03 ± 6.33 mg/dL in hypercholesterolemic diet fed rabbit groups; H, HRC and HRCE, respectively.

HDL-cholesterol

Concerning the diets, F values pertaining to HDL-c (Table 2) expounded non-substantial differences in normal and hypercholesterolemic diet fed rabbits. Means related to the said trait in normal rabbits was 33.51 ± 1.54 (N) that increased by supplementing red cabbage based diets up to 33.64 ± 1.52 mg/dL (NRC) and 34.07 ± 1.92 mg/dL (NRCE). Similarly, hypercholesterolemic rabbits showed an inclining trend from 30.12 ± 1.32 mg/Dl (H) to 31.06 ± 1.73 mg/dL (HRC) and 31.49 ± 1.26 mg/dL (HRCE). Hence, it is evident that red cabbage and its extract raised HDL-c in both dietary patterns but to a minor extent.

Lipidemic ratio

Atherogenic index (AI)

The F values in Table 3 demonstrated that treatments effected significantly on atherogenic index (AI) in both normal and hypercholesterolemic animals. The maximum values were reported in negative & positive control groups for AI 1.18 ± 0.05 & 3.86 ± 0.18, respectively. The treated diets fed to normal rabbits in groups; NRC & NRCE decreased the AI to 1.05 ± 0.06 & 1.06 ± 0.05, accordingly. However, in hypercholesterolemic groups; HRC & HRCE, the marked decrement was observed in AI as 3.00 ± 0.160 & 3.14 ± 0.14.

Cardiac risk ratio (CRR)

The statistical analyses stated that red cabbage based diets impacted significantly on cardiac risk ratio (CRR) in normal as well as hypercholesterolemic animals. The maximum values for CRR were 0.60 ± 0.02 (N) & 2.92 ± 0.17 (H). The treated diets fed to normal rabbits in groups; NRC & NRCE curbed the CRR to 0.55 ± 0.03 & 0.55 ± 0.02, accordingly. However, in hypercholesterolemic groups; HRC & HRCE, CRR showed obvious decrement up to 2.32 ± 0.10 & 2.45 ± 0.09, accordingly.

Table 3 Effect of diets on lipidemic ratio

Experimental groups	Ratio			
	AI	CRR	HTR (%)	AAI
N	1.18 ± 0.05^{a}	0.60 ± 0.02^{a}	45.89 ± 2.63^{b}	0.85 ± 0.04^{b}
NRC	1.05 ± 0.06^{b}	0.55 ± 0.03^{b}	48.67 ± 1.74^{a}	0.95 ± 0.05^{a}
NRCE	1.06 ± 0.05^{b}	0.55 ± 0.02^{b}	48.61 ± 2.31^{a}	0.95 ± 0.04^{a}
F value	19.02**	13.91**	4.94*	17.07**
H	3.86 ± 0.18^{a}	2.92 ± 0.17^{a}	20.59 ± 0.87^{b}	0.26 ± 0.02^{b}
HRC	3.00 ± 0.160^{b}	2.32 ± 0.10^{b}	25.03 ± 1.34^{a}	0.33 ± 0.01^{a}
HRCE	3.14 ± 0.14^{b}	2.45 ± 0.09^{b}	24.13 ± 1.07^{a}	0.32 ± 0.01^{a}
F value	83.96**	60.73**	44.97**	77.65**

Data values represent mean ± SD ($n = 10$); One-way ANOVA followed by Tukey's HSD multiple comparison tests; Means carrying different superscripted letters differ significantly

AI Atherogenic index ((CHOL-HDL-c)/HDL-c), *CRR* Cardiac risk ratio (LDL-c/HDL-c), *HTR* HDL-cholesterol to total cholesterol ratio (HDL-c/CHOL*100), *AAI* Anti-atherogenic index (HDL-c/(CHOL-HDL-c)), *N* Normal diet, *NRC* Normal diet + red cabbage, *NRCE* Normal diet + red cabbage extract, *H* Hypercholesterolemic diet, *HRC* Hypercholesterolemic diet + red cabbage, *HRCE* Hypercholesterolemic diet + red cabbage extract

* = Significant ($p < 0.05$); ** = Highly significant ($p < 0.01$)

HDL-cholesterol to total cholesterol ratio (HTR)

The F values presented significant impact of red cabbage based dietary inclusion on HTR in both normal and hypercholesterolemic animals. The minimum HTR values were reported in normal control group as 45.89 ± 2.63% that increased up to 48.67 ± 1.74% in NRC group and 48.67 ± 1.74% in NRCE group. Mean pertaining to HTR, showed minimum value in hypercholesterolemic diet fed animals i.e. 20.59 ± 0.87%. On feeding red cabbage & administrating its extract, the mentioned trait presented an inclining trend with values reported as 25.03 ± 1.34 & 24.13 ± 1.07%, respectively.

Anti-atherogenic index

The statistical demonstration showed that treatments impacted significantly on AAI in normal as well as hypercholesterolemic animals. Mean pertaining to AAI reported minimum value in normal control group i.e. 0.85 ± 0.04% that raised up to 0.95 ± 0.05% in NRC group and 0.95 ± 0.04% in NRCE group. However, AAI also presented minimum values in positive control oxidative stressed rabbits as 0.26 ± 0.02 that augmented to 0.33 ± 0.01 & 0.32 ± 0.01 as indicated in Table 3.

Briefly, red cabbage fed animals were relatively more protective against atherogenic biomarkers as compared to extract supplemented groups.

Serum oxidative stress biomarkers

Superoxide dismutase (SOD) activity

In response to dietary interventions, SOD depicted non-significant variation in normal rabbits, whereas in

hypercholesterolemic diet fed rabbits, the impact of the treatments on SOD was momentous. Means regarding SOD activity in normal rabbits; N, NRC & NRCE groups were 187.39 ± 9.64, 193.18 ± 9.99 & 192.54 ± 10.35 U/mL. Likewise, red cabbage and its extract raised SOD enzymatic activity in hypercholesterolemic animals, with maximum value as 90.06 ± 3.02 U/mL (HRC) followed by 86.79 ± 4.48 U/mL (HRCE) and 78.53 ± 3.78 U/mL (H). The percent increase in SOD via red cabbage was comparatively higher than red cabbage extract as compared to control is expressed in Fig. 1.

Catalase (CAT) activity

The statistical analysis displayed significant variations in CAT as a function of treatments in both normal and hypercholesterolemic diet fed animals. Means relating to CAT in normal rabbits; N, NRC & NRCE groups were 98.02 ± 4.39, 104.69 ± 5.53 & 100.16 ± 5.13 U/mL, respectively. In hypercholesterolemic animals, similar increment with respect to diets was observed in CAT activity with maximum value reported by HRC group as 74.35 ± 4.26 U/mL as compared to HRCE group as 70.83 ± 3.11 U/mL in contrast to H group as 61.24 ± 2.77 U/mL. Resultantly, red cabbage showed an upper hand in the enhancement of CAT activity as compared to its extract against normal and hypercholesterolemic diet fed groups (Fig. 1).

Lipid peroxidation (MDA)

The F value displayed significant variations in MDA as a function of diets in both normal and hypercholesterolemic diet fed animals. Means regarding MDA (nM/mL) revealed maximum lipid peroxidation in control groups; N and H groups as 6.29 ± 0.24 and 11.02 ± 0.52 that later decreased on provision of red cabbage & red cabbage extract to 5.94 ± 0.24 (NRC) & 6.01 ± 0.23 (NRCE) and 7.95 ± 0.36 (HRC) & 8.66 ± 0.49 (HRCE), correspondingly. Conclusively, red cabbage supplementation presented higher amelioration against lipid peroxidation in normal and hypercholesterolemic diet fed groups as compared to red cabbage extracts administered groups as presented in Fig. 1.

Discussion

The current findings pertaining to lipid lowering ability of cabbage is supported by Sankhari et al. [5]. They found that co-ingestion of extract (100 mg/kg B.W.) plus atherogenic diet (3% cholesterol) to rats for 56-days reduced their cholesterol 57%, triglyceride 23%, LDL 70%, VLDL 27% and atherogenic index 72% however, raised HDL to 32% as compared to atherogenic diet fed control rats. In another animal study, red cabbage powder (10%) and its extract (100 mg/kg B.W.) were tested against paracetamol induced hepatotoxicity. The serum cholesterol, LDL and VLDL were reduced by 38 and 43, 53 and 61 and 29 and 29%, respectively [25]. Likewise, red

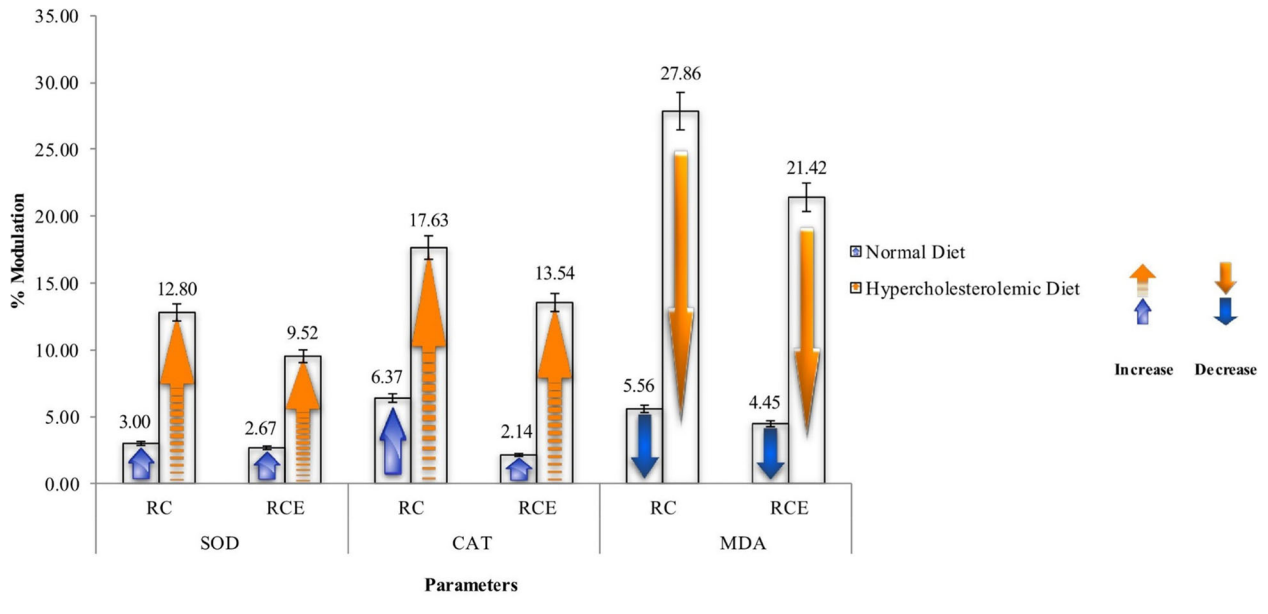

Fig. 1 Percent modulation in serum oxidative stress biomarkers in comparison to control

cabbage powder and its extract was administered to streptozotocin injected rats that reported pronounced decline in cholesterol (32 and 35%), triacylglycerol (32 and 36%), LDL (44 and 46%), VLDL (75 and 75.6%) and MDA (47 and 55%), respectively. However, increase was noted in HDL (32 and 30%) and SOD (47 and 51%) as compared to control animal [26].

Similarly, Al-Dosari [3] conducted an animal trial in which lyophilized red cabbage juice (doses; 250 and 500 mg/kg/day) fed to hypercholesterolemic albino rats for 6 weeks. They reported significant decline in cholesterol, triacylglycerol, LDL and VLDL up to 10 and 33, 16 and 28, 12 and 42, 17 and 29%, whereas HDL showed increase up to 16 and 26% at the corresponding doses. Further investigation revealed that white and red cabbage crude extracts to diabetic rats reduced serum cholesterol (44.4 and 48.09%), triacylglycerol (62.5 and 70.09%), LDL (62.5 & 82.7%) and MDA (41 and 45%). Nevertheless, significant increments in serum HDL and GSH levels were also noticed as compared to positive control group [11].

Regular consumption of red cabbage helps in controlling hyperlipidemia and associated oxidative stress due to the existence of antioxidant vitamins like vitamin C (56% RDA) that defends against inflammatory pathways & chronic cell injury and minerals such as Mn i.e. a cofactor for antioxidant enzyme (MnSOD) in the body [26]. Further, polyphenols in red cabbage like anthocyanins, flavonoids and isothiocyanates as well as ascorbic acid down-regulate HMG-CoA reductase i.e. a rate limiting enzyme in the de novo biosynthesis of cholesterol hence possess hypolipidemic potential [3, 27]. Previous researches reported numerous components in cabbage extract such as hydroxycinnamic acids, flavonoids, β-carotenes, lutein, zeaxanthin, alkaloids, glycosides, saponins, titerpenoids, tannins and polysaccharides. These moieties have proven their hypolipidemic ability in terms of down-regulation of cholesterol 7α-hydroxylase activity; rate-limiting enzyme of bile acid biosynthesis in the liver resultantly enhances fecal excretion of bile acids. Besides, these bioactives are also responsible to lower hepatic gluconeogenesis i.e. directly related to lipogenesis. Thus, any plant extract or medicine that interferes with gluconeogenesis is also responsible to effect on lipogenesis. This mechanistic approach explains cabbage potential to control alterations in lipid profile still further confirmation is required to reach a conclusive approach [2, 27]. Previously, Komatsu et al. [28] also detected a compound "*S*-Methyl-L-Cysteine Sulfoxide" in cabbage extract, involved in cholesterol catabolism and fecal excretion. Another researchers group, Gaafar et al. [11] negatively associated oxidative injury with phenolic acids, vitamin C & α-tocopherol in red cabbage and quercetin & high proportions of isothiocyanates in white cabbage. Additionally, red and black cabbage inclusion in the diet not only enhances fiber but also contain a myriad of bioactive compounds like cyanidin, quercetin, kaempferol, luteolin and vitamin C, responsible to reduce cholesterol absorption, LDL modification and ox-LDL/LDL-c levels to significant levels [10].

Several scientific evidences endorsed the cholesterol lowering ability of anthocyanins. Considering its existence in red cabbage, some studies are described in this regard. Earlier, Kabiri et al. [29], Setorki et al. [30], Asgary et al. [31], Setorki et al. [32] and Ali et al. [33] determined the effect of anthocyanins on cardiac parameters of New Zealand white rabbits in response to high cholesterol (1%) diet. The extract showed significant reductions in Apo-B, cholesterol, triacylglycerol, LDL-c, ox-LDL-c, MDA, AST, ALT, CRP; C-reactive protein and AI however, increments were observed in HDL & Apo-A thus effectively reduced hyperlipidemia. The proposed mechanisms related to hypolipidemic perspectives of anthocyanins include suppression in HMG-CoA reductase, low cholesterol absorption, increase in fecal excretion of cholesterol and bile acids, reduce LDL modification or oxidation by trapping free oxygen radicals such as superoxide anion & per-oxynitrites to protects against protein and lipid oxidation, up-regulation of lipoprotein lipase, reduction in acyl cholesterol acyl transferase i.e. responsible for cholesterol esterification and absorption, secretion of hepatic VLDL, Apo-B & VLDL packaging and less accumulation of triacylglycerol that protects against fatty liver, insulin resistance & obesity [29–32, 34–36]. Furthermore, protocatechuic acid, a breakdown molecule of anthocyanin, possesses high antioxidant activity as compared to anthocyanins and fights against atherogenesis and oncogenesis [37].

In context to the current study, the whole cornelian cherry fruit and its purified anthocyanin fraction was tested on cholesterol 1% administered rabbits by Sozański et al. [38]. The cornelian cherry (100 mg/kg/day) stated marked decrease in MDA, resultantly preserved hepatic glutathione level. On the other hand, pure anthocyanins from cornelian cherry significantly lowered lipid profile and formation of foam cells in the experimental animals. Another investigation by Finné Nielsen et al. [34] compared purified anthocyanin from black currants and black currant juice on hyperlipidemic rabbits. They found increment in LDL and cholesterol via purified antioxidants though an inverse response was noted in black currant juice without any adverse effects. The study also depicted improvement in antioxidant enzymes; SOD and CAT in control (1338 ± 108 and 9.21 ± 1.54 U/g hemoglobin), purified anthocyanins (1401 ± 162 and 9.97 ± 1.04 U/g hemoglobin) and black currant juice supplemented groups (1490 ± 148 and

9.17 ± 1.97 U/g hemoglobin). Conclusively, anthocyanins showed higher absorption through juice than purified anthocyanins. Further, they reported similarities in absorption and excretion of anthocyanins in rabbits and humans.

Additionally, Yanni et al. [39] studied the synergistic role of varied antioxidant moieties in whole fruit (corinthian currant; 10%) on hypercholesterolemic New Zealand white rabbits for 8 weeks. In corinthian currant, gallic acid, catechins and epicatechin inhibit pancreatic cholesterol esterase or bind bile acids resultantly delay cholesterol absorption. Apart from this, metabolites of flavonoids may have antioxidant activity within the physiological system. Besides, the effect of dietary fiber in the plant matrix i.e. cocoa (165 g/kg) was studied in hypercholesterolemic rats during 3 wk. period by Lecumberri et al. [40]. The outcomes of the study mentioned reduction in LDL-c and restoration of triacylglycerol to normal levels, whereas no impact was noticed on serum antioxidant enzymes. Recently, molecular level studies have shown that fatty foods are responsible for alterations in transcription factors like PPAR-γ, Nrf2, NF-κB and SREBP-1c in liver and adipose tissues however, their modulatory responses have been observed via polyphenol rich diet. In future, these expressions also need to be investigated by supplementing cabbage polyphenols [41, 42].

Conclusions

The whole red cabbage fed to rabbits demonstrated an upper hand in modulating serum lipidemic parameters and oxidative stress biomarkers as compared to red cabbage extract. This response could be linked with fiber content or synergistic impact of bioactives in whole plant matrix that might get lost or oxidized during extraction process. This notion requires further confirmation by new researchers in this field. In the nutshell, incorporation of red cabbage in dietary regimen has proven as an effective approach in lowering hypercholesterolemia and related oxidative stress. Domestically, such studies might go a long way in guiding vegetables growers to promote red cabbage production.

Abbreviations

AAI: Anti-atherogenic Index; AI: Atherogenic Index; CAT: Catalase; CHD: Coronary heart disease; CRP: C-reactive protein; CRRs: Cardiac Risk Ratios; HDL: High density lipoprotein; HTR: HDL to total cholesterol ratio; LDL-c: Low density lipoprotein-cholesterol; non-HDL-c: Non- high density lipoprotein-cholesterol; ROM: Reactive Oxygen Metabolites; ROS: Reactive Oxygen Species; SOD: Superoxide dismutase; TAG: Triacylglycerol; TBARS: Thiobarbituric acid reactive substances; TC: Total cholesterol; VLDL-c: Very low density lipoprotein-cholesterol

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Authors' contributions

FA designed the project under the supervision of MSB and AB helped in conducting animal trials. FA, ST and HARS prepared the final manuscript. All authors read and approved the final manuscript.

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Consent for publication

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Competing interests

The authors declare that they have no competing interests.

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