Fishery Technology 58 (2021) : 219 - 228

Cardio-protective effect of Thiamine and Pyridoxine loaded Vanillic acid grafted Chitosan micro-particles against Isoproterenol-induced myocardial Infarction in animal model

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Abstract

In the present study, the cardio-protective effect of dietary supplementation of thiamine and pyridoxine loaded vanillic acid grafted chitosan microparticles (TPVGC) on isoproterenol induced myocardial infarction in Wistar strain albino rats were studied. Animals were distributed into four experimental groups which were fed at graded level of levels TPVGC for 45 days *viz.,* Control (C) (basal feed + 0% TPVGC); T1 (basal feed + 0.8% TPVGC); T2 (basal feed + 1.6% TPVGC); T3 (basal feed + 2.4% TPVGC) for experimental period of 45 days. The possible role of dietary TPVGC on cardioprotective effect was assessed in terms of Serum LDH, IgG, Homocysteine, Serum Myglobin, troponin–T, CPK-MB, S-GOT, S-GPT, and other metabolic enzyme assays. All the assessed parameters were found to have significant difference between the control and treatment groups. The biochemical markers such as troponin–T, Serum LDH, CPK-MB, S-GOT, S-GPT, IgG, Homocysteine, Serum Myglobin exhibited significant ($p<0.05$) difference between the control and treatment groups. Moreover, metabolic and antioxidant enzyme activity in the tissue levels also showed significant ($p<0.05$) difference between the control and treatment groups. Results from the present study, revealed that dietary supplementation of thiamine and pyridoxine loaded vanillic acid grafted chitosan microparticles at a minimum level of 1.6% have protective effect on experimental myocardial infarction induced by isoprenaline in rats.

Keywords: Vitamins, chitosan, vanillic acid, cardioprotection, myocardial infarction

Introduction

In the recent days, myocardial infarction has become the most common prevailing disease in India and across the globe as well. Worldwide, on a yearly basis, nearly, 17.3 million deaths have been recorded and it is anticipated to grow to more than 23.6 million by 2030, The cause of death being cardiovascular disease (Mozaffarian et al., 2015). In line with western countries, India also witnessed the increase of cardiovascular disease, the main risk factors identified being tobacco consumption, physical inactivity, eating habits, lack of awareness and treatment to control cardiovascular disease (Thankappan et al., 2010). In the recent past, researchers around the globe have reported the possible correlation between the individual nutritional factors and chronic diseases, with a special reference to cardiovascular disease. Recently, nutraceuticals or novel food supplements/ingredients that are having nutrient interaction in the food matrix have gained attention to combat the chronic diseases (Neufcourt et al., 2016; Hu, 2002; Jacobs et al., 2009). Zheng et al. (2012) have reported that consumption of seafood is directly correlated to reduce vascular diseases and certain cancers.

Chitosan, a natural biopolymer, known for its bioactive properties has proven useful in various applications such as agriculture, food and pharmaceutical sectors. Earlier studies conducted in our

Received 10 August 2021; Revised 30 October 2021; Accepted 05 October 2021

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laboratory have reported that dietary supplementation of chitosan was found have antiulcer effect against artificially induced peptic ulcer in rats and found to very efficient in protecting the heart tissue against the isoprenaline-induced toxicity (Anandan et al., 2012). The positive health beneficial effect of vanillic acid has been reported by the scientific communities across the globe. The effective immunomodulatory role of vanillic acid has been reported by Chiang et al. (2003). Earlier studies conducted by many of the other researchers have shown that vanillic acid exhibits anti-filarial, anti-bacterial, antimicrobial properties (Varma et al., 1993; Rai et al., 1966; Delaquis et al., 2005). Tsuda et al. (1994) reported the chemo preventive effect of various antioxidant substance along with vanillin in animal model. Prince et al. (2011) reported the health beneficial aspect of vanillic acid in terms of cardioprotective supplement against the myocardial infarcted in experimental animals. Dianat et al. (2016) reported that supplementation of vanillic acid through oral administration has cardioprotective effect in rat. The health and health care beneficial effects of chitosan and vanillic acid are studied extensively, however, the combination of these two compounds and its role on cardioprotective effect has not been investigated.

Isoproterenol (ISO) is a commonly used drug to induce myocardial infarction in the experimental animal model. It is a combination of catecholamine, and β-adrenergic agonist, known to influence adverse effect in heart, in terms of biochemical, functional and structural changes (Shiny, et al., 2005; Goyal et al., 2010). Having the above said background, the study was aimed to assess the potential role of supplementation of thiamine and pyridoxine loaded vanillic acid grafted chitosan micro-particles (TPVGC) on cardioprotective effect against the isoprenaline-induced myocardial infarction in rats.

Materials and Methods

Thiamine and pyridoxine-loaded vanillic acid grafted-chitosan acid grafted chitosan was synthesized as reported earlier (Tejpal et al. 2017). Animals, weighing 200-230 g were chosen for the experiment. Animals selected for the study were housed in cages, and hygienically kept under suitable environmental conditions (temperature, 28±2°C; 12 h light/dark cycle; humidity, 60–70%) and fed with standard diet (Krish Feeds, Bangalore, India) and water *ad libitum*.

After acclimatization for seven days, twenty-four animals were randomly arranged into four experimental groups. The animals were fed with a diet containing 0, 0.8, 1.6 and 2.4% of thiamine and pyridoxine-loaded vanillic acid grafted-chitosan acid grafted chitosan during the experimental period. The experiment consists of four groups such as control (animals fed with standard diet with 0% TPVGC); T_1 (animals fed with standard diet with 0.8% TPVGC); T_2 (animals fed with standard diet with 1.6% TPVGC); T_3 (animals fed with standard diet with 2.4% TPVGC). The experiment was conducted by following the guidelines laid by institute CPSCEA approved animal ethics committee.

At the completion of 45 days feeding trial, cardioprotective efficiency of TPVGC was assessed by artificially inducing myocardial infarction to experimental animals by isoprenaline injection through intraperitoneal (i.p) @ 11 mg per 100 g body weight / day, for two consecutive days (Anandan et al., 2012).

After completing trials, animals were sacrificed by means of chloroform anaesthesia. The blood was collected and allowed to clot under controlled condition. Finally, the serum was collected by removing the clot by centrifugation. For enzyme assay, different tissue such as liver, muscle, kidney, and heart were homogenized with 0.25 M chilled sucrose solution. Homogenate was subjected to centrifugation (10000 rpm at 4°C for 10 min) and supernatants was collected for enzyme assays and kept $^{-}$20°C. Protein content in the tissue was determined by following the method given by Lowry et al. (1951).

CPK-MB in serum was estimated using the assay kit procured from Agappe Diagnostics (Kerala, India). Serum GOT, GPT and LDH activity were analyzed by colorimetric method assay kit (Origin). Troponin T in serum sample was estimated by following the protocol given by Farvin et al. (2009) using immunoassay analyzer. Commercial kit (Elabscince assay kit) was used to assess the homocysteine content in serum sample. Serum myoglobin levels were measured by ELISA assay kit procured from Sigma-Aldrich. Homocysteine in serum was estimated by assay kit procured from Elabscince. Serum sample from the experimental animals were subjected to lipid profile to check the content of cholesterol, high density lipo-protein (HDL), low

density lipo-protein (LDL) and very low density lipo-protein (VLDL) and triglycerides (TG), the analysis was done using an auto analyzer.

The Aspartate amino transferase (AST) and Alanine amino transferase (ALT) activities in the tissue samples were estimated by adopting the method as described by Wooten (1964). LDH and MDH activities were measured by the change in optical density (OD) at 340 nm for 5 min using the method of Wrobleuiski & Ladue (1955) and Ochoa (1955), using sodium pyruvate and Oxalo-acetate were used as substrates, respectively. Antioxidant enzyme, SOD (Superoxide Dismutase) expression was assayed in the tissues of experimental animal by following the protocol described by Misra & Fridovich (1972). The catalase enzyme activity was measured in tissues of experimental animals by following the method given by Claiborne (1985).

The data pertaining cardioprotective study was subjected to one way ANOVA and statistical significance between the mean was tested by using Duncan's multiple range. The comparisons were made at the 5% probability level and regression analysis was done. Statistical software package SPSS (version 16) was used to carry out the analyses.

Results and Discussion

The troponin–T content in the serum sample of the experimental animals varied significantly ($p<0.05$) with respect to the control and treatment group (Fig. 1). Animals fed without the TPVGC in the diet have recorded higher troponin–T value, whereas the dietary supplementation of TPVGC had influenced positively and significantly reduced the troponin–T content and animal group fed with 2.4% TPVGC recorded lower level of troponin–T. Present study reveals that levels of troponin T in the serum samples has increased notably in the control group, certainly due the degree of damage in the heart, which was induced by Isoproterenol. Troponin T is the most common indicator for diagnosing the myocardial infarction. The study was in line with the finding of Huang et al. (2018) stating that the injecting scutellarin at 40 mg kg^{-1} has reduced the degree of myocardial infraction in rats. Generally, troponins I and T are used as specific markers to identify the myocardial injury. Acikel et al. (2005) reported higher levels of troponin T was recorded in the myocardial infarcted rats and animal treated with dantrolene was found to reduce level of troponin T. Similarly, Priscilla et al. (2009) who reported that gallic acid supplementation at graded levels found to have cardioprotective effect against the myocardial infarcted experimental animals.

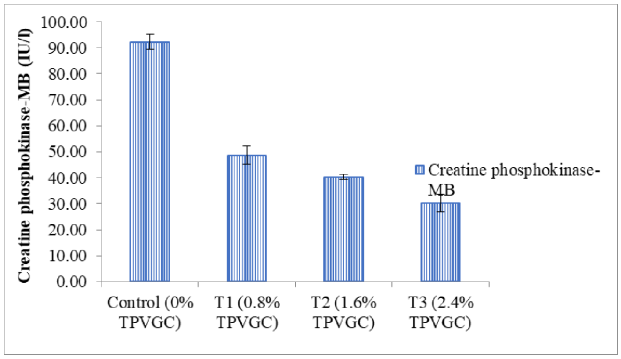

Fig. 1. Effect of TPVGC supplementation on Troponin–T activity in experimental animals

Troponin values are expressed as mean±SE (n=6)

All the cardiovascular disease indicating enzymes like, CPK-MB, AST, ALT and lactate dehydrogenase activities in the serum sample varied significantly among the experimental group compared to control (Fig. 2, 3 and 4). Elevated level of cardiovascular marker enzymes were observed in control group, and dietary supplementation of TPVGC at different concentration has recorded gradual reduction in the marker enzymes. Animals fed at 2.4% of TPVGC in the diet has recorded lowest level of marker enzymes. Serum CPK-MB, AST, ALT, and LDH are the well-established indicators for diagnosing myocardial infraction in animal and human as well. Generally, during the process of myocardial infraction or cardiovascular disease and adverse conditions, myocardial cells get damaged and the marker enzymes start leaching in to the blood stream (Mathew et al., 1985). The results of the present study shows that the dietary supplementation of TPVGC has positively influenced reduction of these bio-marker enzymes in the serum sample. This effect may be associated with the combined impact of water soluble vitamins (thiamine and pyridoxine) and the vanillic acd grafted chitosan. The present study is in line with the findings of Priscilla et al. (2009) who reported that administration of phenolic compound (gallic acid) has greatly reduced the activity of cardiovascular disease indicating enzymes in experimental animals. Dietary supplementation of folic acid in combination with Vitamin B_{12} was found to protect animals from myocardial infarction induced through isoproterenol injection (Hagar, 2002).

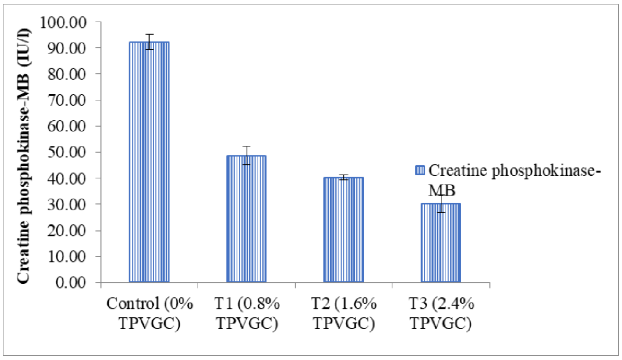

Fig. 2. Impact of dietary supplementation of TPVGC on Creatine phosphokinase-MB activity in experimental animals

CPKMB values are expressed as mean±SE (n=6)

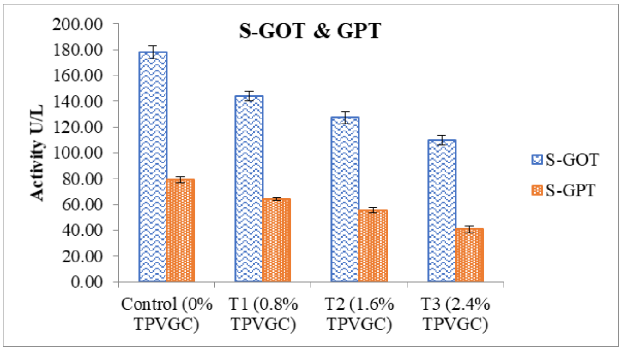

Fig. 3. Influence of dietary supplementation of TPVGC on S-GOT & GPT activity in experimental animals

Enzyme values are expressed as mean±SE (n=6)

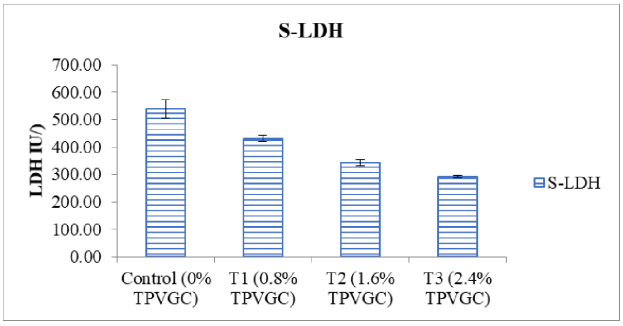

Fig. 4. Influence of dietary supplementation of TPVGC on S-LDH activity in experimental animals

Serum LDH values are stated as mean ± SE (n= 6)

Both myoglobin and homocysteine levels in serum samples were significantly ($p < 0.05$) higher in the control compared to treatment group (Fig. 5 and 6). Incremental supplementation of TPVC in the diet reduced the serum myoglobin and homocysteine and lowest levels were recorded in the animals fed at 2.4%. Myoglobin is a protein molecule having heterodimer which consists of peptide chain and heme group. This particular protein is found in both skeletal and myocardial muscle cells and during the muscle damage the myoglobin is leached in to blood streams. Generally, elevated level of serum myoglobin represents the potential prognostic risk towards cardiovascular disease. The myoglobin is used as biomarker for diagnosing myocardial infraction (Yao et al., 2016; Li et al., 2020). Results of the present study exhibited that dietary supplementation of TPVGC in the treatment group reduced the serum myoglobin levels compared to control group. Similarly, Stanely et al. (2020) reported that oral administration of sinapic acid could act as a mechanism to protect cardiac mitochondria from the isoprenaline induced myocardial infarcted experimental animals (Stanely et al., 2020). Soccer players supplemented with L-carnitine-L-tartrate could reduce myoglobin levels leading to less fatigue (Naclerio et al., 2015). Similar to myoglobin, homocysteine also followed similar trend. Higher amount of homocysteine in the blood and myocardial tissue can be correlated to cardiovascular complications. It is also considered as one of the sensitive biomarkers for the cardiovascular disease or myocardial infraction (Qujeq et al., 2001). The present study can be linked with the findings that folic acid and vitamin B_{12} supplementation played crucial role in reducing the homocysteine in blood showing cardioprotective effect (Hagar, 2002). Further, cardiovascular disease can be correlated with higher serum myoglobin and homocysteine with elevated levels of cholesterol, triglyceride, LDL and VLDL.

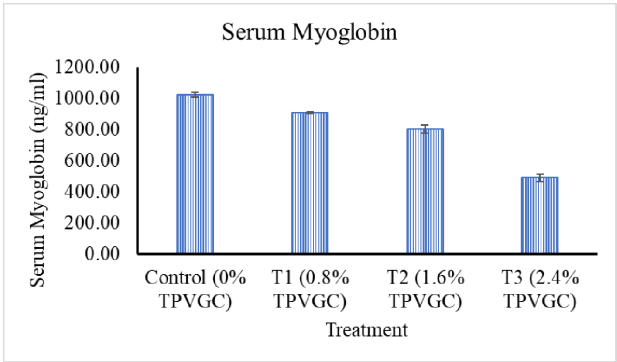

Fig. 5. Impact of dietary supplementation of TPVGC on Serum Myoglobin activity in experimental animals

Values of Serum Myoglobin are expressed as mean±SE

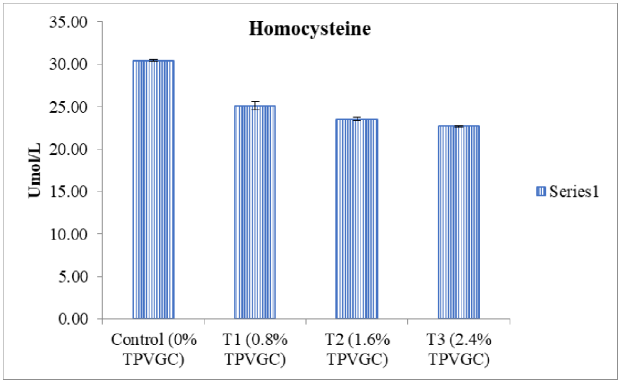

Fig. 6. Impact of dietary supplementation of TPVGC on homocysteine activity in experimental animals

Values are expressed as mean±SE

Serum Lipid profile of the experimental animals showed significant ($p<0.05$) difference among the control and treatment group (Table 1). Highest levels of serum lipid profile in terms of cholesterol, LDL, VLDL and triglycerides were recorded in the control group fed without TPVGC in the diet, however, TPGVC supplemented group showed an incremental decrease. Lowest levels are recorded in the experimental animals fed with 2.4% of TPVGC in the diet, whereas, reverse trend was observed in HDL. Animal fed with 2.4% of TPVGC has recoded highest HDL and lowest was in the control group. Dyslipdemia is an abnormal amount of lipids content in the blood, is the key risky factor associated with the cardiovascular disease (Fuster &

Table 1. Effect of dietary supplementation of TPVGC on Serum lipid profile in the experimental animal

Parameters	Treatments			
	Control (0% TPVGC)	T_1 (0.8% TPVGC)	T_2 (1.6% TPVGC)	T_3 (2.4% TPVGC)
Serum Cholesterol	100.00^{d}±0.58	95.50^{c}±0.29	85.27^{b}±0.65	83.08^{a}±0.58
Serum Triglycerides	111.67^{d}±0.88	96.00^{c}±0.58	88.00^{b}±0.58	84.00^{a}±0.58
Serum HDL	25.33^{a}±0.88	27.33^{a}±1.20	32.33^{b}±0.88	37.03^{c}±0.58
Serum LDL	54.33^{c}±2.33	46.33^{c}±0.88	41.67^{b}±0.88	31.33^{a}±1.70
Serum VLDL	24.00^{c}±0.58	20.33^{b}±0.58	17.00^{a}±0.58	16.00^{a}±1.53

Different superscript in the same row indicate statistical significance ($p<0.05$) between the control and treatment groups (control, T_1, T_2 and T_3). Values are expressed as mean±SE (n=3). Units: mg dl^{-1}

Table 2. Impact of dietary supplementation of TPVGC on the activities of AST and ALT activity in heart, liver, kidney and muscle tissue of myocardial infarcted rats.

Parameters	Tissue	Treatments			
		Control (0% TPVGC)	T_1 (0.4% TPVGC)	T_2 (0.8% TPVGC)	T_3 (1.6% TPVGC)
AST	Heart	3.16^{b}±0.53	2.86^{b}±0.71	1.70^{ab}±0.38	0.72^{a}±0.14
	Liver	7.34^{c}±0.32	5.69^{b}±0.39	5.09^{b}±0.67	3.45^{a}±0.67
	Kidney	6.38^{c}±0.36	4.80^{b}±0.18	4.65^{b}±0.06	3.60^{a}±0.12
	Muscle	30.24^{b}±1.48	26.29^{b}±1.01	23.99^{ab}±1.17	22.39^{a}±1.48
ALT	Heart	30.18^{a}±2.53	15.45^{a}±0.35	15.57^{a}±0.31	16.07^{a}±0.19
	Liver	11.10^{b}±0.56	8.52^{a}±0.43	8.73^{a}±0.69	7.58^{a}±0.38
	Kidney	28.62^{b}±1.61	13.73^{a}±0.34	13.07^{a}±0.09	14.88^{a}±0.89
	Muscle	33.22^{b}±2.10	30.80^{b}±1.27	28.18^{b}±1.38	20.92^{a}±2.30

Different superscripts in the same row indicate statistical significance ($p<0.05$) between the control and treatment groups (control, T_1, T_2 and T_3). Values are expressed as mean±SE (n=6).

Units:AST- nano moles oxalo acetate released/ mg protein/ minute at 37°C; ALT- nano moles of sodium pyruvate formed/ mg protein/minute at 37°C

Kelly, 2010). High levels of cholesterol in the blood and cholesterol accumulation in the heart tissue can be directly linked with myocardial infarction (Salter & White, 1996). Similarly, higher levels of triglyceride, LDL, VLDL and reduced levels of high density lipoproteins also are associated with cardiovascular disease (Munshi et al., 2014; Prenner et al., 2014). Results of the present study show that supplementation of TPGVC has significantly reduced the cholesterol, triglyceride, LDL and VLDL and enhanced the high density lipoproteins levels. This could be due to the combined effect of water soluble vitamins and vanillic acid grafted chitosan. The results are in agreement with findings of Jiang et al. (2019) which revealed the effect of dietary supplementation of chitosan oligosaccharides on cardioprotective effect. Dietary supplementation of pyridoxine is proportionately linked with decreased risk of cardiovascular disease (Jeon et al., 2019). Likewise, Kakadiya et al. (2010) have also reported that hesperidin (byproduct of citrus) administration has reduced the total cholesterol, triglyceride, LDL and VLDL.

Metabolic enzyme (AST & ALT) activities in the tissues of the experimental animals displayed significant difference ($p<0.05$) (Table 2). Higher enzyme levels were observed in the control group compared to the animal fed with graded levels of vitamin B_1 and B_6 loaded vanillic acid cross-linked chitosan in the diet. Enzyme, LDH and MDH levels in the respective tissues showed similar trend of AST and ALT (Table 3). The results support the findings that dietary supplementation of tocopherol alone and also in combination with lycopene was found to have lower levels of AST and ALT activity compared to the isoproterenol-induced myocardial infarction group (Upaganlawar et al., 2010). Ganesan et al. (2010) has reported that oral administration of betaine in the experimental group recorded lower levels of AST and ALT activity. Dietary supplementation of gallic acid was found to lower the AST and ALT activity and was proven to have cardioprotective effect (Priscilla et al., 2009). Similarly, dietary supplementation of red palm oil has reduced the LDH activity in the heart tissue and also reduced the myocardial infarct size in animals (Bester et al., 2010). The supplementation of sardine oil loaded vanillic acid cross-linked chitosan was also found to lower LDH and MDH activity in rat tissues (Vishnu et al., 2018).

Supplementation of TPVGC in the treatment group showed significantly ($p<0.05$) lower antioxidant enzyme (SOD, catalase and Gluthathione-S-transferase) activity compared to control group (Table 4). Antioxidant enzymes act as front line defence mechanism against the increased superoxide production in the living system. As per the earlier report, oxidative stress impacts in cardiac restoration. Generally, elevated levels of oxidative stress and cardiac oxidation have been linked to diastolic heart failure (Madamanchi & Runge, 2013; Murino Rafacho et al., 2017). In the present study, the reduction in the antioxidant enzyme activity may be liked with dietary supplementation of TPVGC

Table 3. Effects of dietary supplementation of TPVGC on LDH and MDH activity in heart, liver, kidney and muscle tissue of myocardial infarcted rats.

Parameters	Tissue	Treatments			
		Control (0% TPVGC)	T_1 (0.4% TPVGC)	T_2 (0.8% TPVGC)	T_3 (1.6% TPVGC)
LDH	Heart	$7.01^{c}\pm0.45$	$4.23^{a}\pm0.21$	$4.12^{a}\pm0.28$	$5.58^{b}\pm0.32$
	Liver	$3.24^{b}\pm0.11$	$2.86^{b}\pm0.09$	$2.90^{b}\pm0.18$	$2.18^{a}\pm0.18$
	Kidney	$6.24^{d}\pm0.17$	$3.55^{c}\pm0.23$	$1.44^{b}\pm0.09$	$2.63^{a}\pm0.29$
	Muscle	$10.39^{b}\pm0.84$	$8.19^{a}\pm0.49$	$7.85^{a}\pm0.34$	$6.32^{a}\pm0.63$
MDH	Heart	$59.33^{b}\pm3.29$	$36.35^{a}\pm1.15$	$36.17^{a}\pm3.32$	$40.41^{a}\pm1.89$
	Liver	$34.58^{c}\pm0.66$	$28.84^{b}\pm0.31$	$27.93^{b}\pm1.73$	$22.27^{a}\pm0.80$
	Kidney	$59.67^{b}\pm4.03$	$33.94^{a}\pm0.31$	$32.70^{a}\pm0.09$	$31.46^{a}\pm2.18$
	Muscle	$99.73^{b}\pm5.50$	$85.48^{ab}\pm4.19$	$82.32^{ab}\pm7.71$	$75.94^{a}\pm5.40$

Different superscripts in the same row indicates significant difference ($p<0.05$) among the control and treatment groups. Units: nanomoles of pyruvate utilized/mg protein/min (LDH); nanomoles of Oxaloacetate utilized/mg protein/min (MDH)

Table 4. Effects of dietary supplementation of TPVGC on SOD, Catalase and GST activity in heart, liver, kidney and muscle tissue of myocardial infarcted rats.

Parameters	Tissue	Treatments			
		Control (0% TPVGC)	T_1 (0.4% TPVGC)	T_2 (0.8% TPVGC)	T_3 (1.6% TPVGC)
SOD	Heart	114.03^{a}±7.66	102.33^{a}±7.28	100.02^{a}±4.09	64.83^{a}±2.15
	Liver	149.30^{a}±2.42	153.03^{a}±9.84	101.14^{a}±7.26	106.73±4.10
	Kidney	98.95^{c}±7.76	99.57^{b}±5.11	96.06^{b}±5.16	74.56^{a}±4.74
	Muscle	61.84^{a}±0.84	59.49^{a}±3.99	62.53^{a}±2.95	61.95^{a}±2.69
Catalase	Heart	19.28^{c}±0.46	15.41^{b}±0.27	11.50^{a}±1.66	10.81^{a}±1.09
	Liver	64.88^{b}±1.75	56.02^{b}±1.09	53.64^{a}±2.33	46.13^{a}±0.87
	Kidney	53.23^{b}±2.04	52.95^{b}±0.90	43.85^{b}±0.75	40.51^{a}±0.83
	Muscle	28.10^{b}±4.34	6.40^{b}±0.55	8.54^{a}±0.74	4.08^{a}±0.50
GST	Heart	0.10^{c}±0.01	0.08^{c}±0.01	0.05^{b}±0.01	0.03^{a}±0.01
	Liver	0.68^{a}±0.01	0.61^{a}±0.01	0.34^{a}±0.23	0.42^{a}±0.04
	Kidney	0.10^{c}±0.01	0.07^{b}±0.01	0.04^{ab}±0.01	0.04^{a}±0.01
	Muscle	0.14^{c}±0.01	0.13^{bc}±0.01	0.11^{b}±0.01	0.08^{a}±0.01

Different superscripts in the same row indicate statistical significance ($p<0.05$) between the control and treatment groups (control, T_1, T_2 and T_3). Values are expressed as mean±SE (n=6).

Units: Catalase, m mol H_2O_2 decomposed min^{-1} mg^{-1} protein at 37 ºC ; SOD, 50% inhibition of epinephrine auto oxidation mg^{-1} protein min^{-1} and GST, μ moles of CDNB-GSH conjugate formed min^{-1} mg^{-1} protein.

which is a combination of thiamine, pyridoxine and vanillic acid grafted chitosan and is directly associated with change in the energy metabolism by reducing the oxidative stress induced though Isoproterenol. The TPVGC can act as impersonator for the antioxidant enzymes in the system. Results of the present work are in agreement with findings of Murino Rafacho et al. (2017) who reported that dietary supplementation of rosemary reduced the oxidative stress and also protected from myocardial infraction. Dietary intake of fruits and vegetables was found to increase the antioxidants concertation in the blood and reduced the oxidation of cholesterol (Zino et al., 1997; Asplund, 2002; Liu et al., 2018). Presence of antioxidants in the living system generally acts as a non-enzymatic antioxidant mechanism. Studies have also reported the beneficial effect of vitamin supplementation on reduction of cardiovascular disease (Riccioni et al., 2007).

In the recent past, change in lifestyle and food habits of human beings has led to increased risk of cardiovascular disease. Cardioprotective nutrients / dietary supplements are very much necessary to combat the cardiovascular disease. Water soluble vitamins, B_1 and B_6 are found to contribute towards the maintenance of cardiovascular health. But, water soluble vitamins are flushed out of the system very quickly. Hence, in the present study, vitamin B_1 and B_6 were encapsulated with vanillic acid cross-linked chitosan. Vanillic acid and chitosan are known for their bio-functional properties. The combination of dietary thiamine, pyridoxine, vanillic acid and chitosan was found to have cardioprotective effect on myocardial infarction induced by isoprenaline in rat model and hence, vitamin B_1 and B_6 loaded vanillic acid cross-linked chitosan can be used as bio-functional product to combat the cardiovascular disease.

Acknowledgements

The authors gratefully acknowledge the Director, ICAR-Central Institute of Fisheries Technology, Cochin for providing the necessary facilities.

References

Acikel, M., Buyukokuroglu, M.E., Erdogan, F., Aksoy, H., Bozkurt, E. and Senocak, H. (2005) Protective effects of dantrolene against myocardial injury induced by isoproterenol in rats: biochemical and histological findings. Int. J. Cardiol. 98: 389-394

Anandan, R., Ganesan, B., Obulesu, T., Mathew, S., Kumar, R. S., Lakshmanan, P. T and Zynudheen, A. A. (2012) Dietary chitosan supplementation attenuates isoprenaline-induced oxidative stress in rat myocardium. Int. J. Biol. Macromol. 51: 783-787

Asplund, K. (2002) Antioxidant vitamins in the prevention of cardiovascular disease: a systematic review. J. Intern. Med. 251: 372-392

Bester, D. J., Kupai, K., Csont, T., Szucs, G., Csonka, C., Esterhuyse, A. J., Ferdinandy, P. and Van Rooyen, J. (2010) Dietary red palm oil supplementation reduces myocardial infarct size in an isolated perfused rat heart model. Lipids Health Dis. 9: 1-9

Chiang, L. C., Ng, L. T., Chiang, W., Chang, M. Y. and Lin, C. C. (2003) Immunomodulatory activities of flavonoids, monoterpenoids, triterpenoids, iridoid glycosides and phenolic compounds of *Plantago* species. Planta medica. 69: 600-604

Claiborne, A. (1985) Catalase activity In: Handbook of Methods for Oxygen Free Radical Research (Greenwald, R. A., Ed). CRC Press, Boca Raton, Florida, pp 283-284

Delaquis, P., Stanich, K. and Toivonen, P. (2005) Effect of pH on the inhibition of Listeria spp. by vanillin and vanillic acid. J. Food Prot. 68: 1472-1476

Dianat, M., Hamzavi, G. R., Badavi, M. and Samarbafzadeh, A. (2015) Effect of vanillic acid on ischemia-reperfusion of isolated rat heart:Hemodynamic parameters and infarct size assays. Indian J. Exp. Biol. 53: 641-646

Farvin, K. H. S., Anandan, R., Kumar, S. H. S., Mathew, S., Sankar, T. V. and Nair, P. G. V. (2009) Biochemical studies on the cardioprotective effect of squalene against isoprenaline-induced myocardial infarction in rats. Fish. Technol. 46: 139-151

Fuster, V. and Kelly, B. B. (2010) Promoting Cardiovascular Health in the Developing World: A Critical Challenge to Achieve Global Health (eds Valentín Fuster & Bridget B. Kelly). The National Academies Press

Ganesan, B., Buddhan, S., Anandan, R., Sivakumar, R. and Anbin Ezhilan, R. (2010) Antioxidant defense of betaine against isoprenaline-induced myocardial infarction in rats. Mol. Biol. Rep. 37: 1319-1327

Goyal, S., Arora, S., Bhatt, T.K., Das, P., Sharma, A., Kumari, S. and Arya, D.S. (2010) Modulation of PPAR- by telmisartan protects the heart against myocardial infarction in experimental diabetes. Chem. Biol. Interact. 185: 271-280

Hagar, H. H. (2002) Folic acid and vitamin B12 supplementation attenuates isoprenaline-induced myocardial infarction in experimental hyperhomocysteinemic rats. Pharmacol. Res. 46: 213-219

Hu, F. B. (2002) Dietary pattern analysis: a new direction in nutritional epidemiology. Curr. Opin. Lipidol. 13: 3-9

Huang, H., Geng, Q., Yao, H., Shen, Z., Wu, Z., Miao, X. and Shi, P. (2018) Protective effect of scutellarin on myocardial infarction induced by isoprenaline in rats. Iran. J. Basic Med. Sci. 21: 267

Jacobs, D. R. Jr., Gross, M. D. and Tapsell, L. C. (2009) Food synergy: an operational concept for understanding nutrition. Am. J. Clin. Nutr. 89: 1543S-1548S

Jeon, J. and Park, K. (2019) Dietary vitamin B6 intake associated with a decreased risk of cardiovascular disease: a prospective cohort study. Nutrients. 11: 1484

Jiang, T., Xing, X., Zhang, L., Liu, Z., Zhao, J. and Liu, X. (2019) Chitosan oligosaccharides show protective effects in coronary heart disease by improving antioxidant capacity via the increase in intestinal probiotics. Oxid. Med. Cell. Longevity. 2019: 1-11

Kakadiya, J., Mulani, H. and Shah, N. (2010) Protective effect of hesperidin on cardiovascular complication in experimentally induced myocardial infarction in diabetes in rats. J. Basic Clin. Pharmacy. 1: 85

Li, X., Yan, H., Zhang, X., Huang, J., Xiang, S. T., Yao, Z., Zang, P., Zhu, D., Xiao, Z. and Lu, X. (2020) Elevated serum myoglobin levels at hospital admission and the risk of early death among patients with hemophagocytic lymphohistiocytosis: evidence from 155 pediatric patients. Ann. Hematol. 99: 963-971

Liu, Z., Ren, Z., Zhang, J., Chuang, C.C., Kandaswamy, E., Zhou, T. and Zuo, L. (2018) Role of ROS and nutritional antioxidants in human diseases. Front. Physiol. 9: 477

Madamanchi, N. R. and Runge, M. S. (2013) Redox signaling in cardiovascular health and disease. Free Radic. Biol. Med. 61: 473-501

Mathew, S., Menon, P. V. and Kurup, P. A. (1985) Effect of administration of vitamin A, ascorbic acid and nicotinamide adenine dinucleotide+ flavin adenine dinucleotide on severity of myocardial infarction induced by isoproterenol in rats. Indian J. Exp. Biol. 23: 500

Misra, H. P. and Fridovich, I. (1972) The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem. 247: 3170-3175

Mozaffarian, D., Benjamin, E. J., Go, A. S., Arnett, D. K., Blaha, M. J., Cushman, M., De Ferranti, S., Després, J. P., Fullerton, H. J., Howard, V. J. and Huffman, M. D. (2015) Executive summary: heart disease and stroke statistics—2015 update: a report from the American Heart Association. Circulation. 131: 434-441

Munshi, R. P., Joshi, S. G. and Rane, B. N. (2014) Development of an experimental diet model in rats to

study hyperlipidemia and insulin resistance, markers for coronary heart disease. Indian J. Pharmacol. 46: 270

Murino Rafacho, B. P., Portugal dos Santos, P., Gonçalves, A. D. F., Fernandes, A. A. H., Okoshi, K., Chiuso-Minicucci, F., Azevedo, P. S., Mamede Zornoff, L. A., Minicucci, M.F., Wang, X.D. and Rupp de Paiva, S.A. (2017) Rosemary supplementation (*Rosmarinus oficinallis* L.) attenuates cardiac remodeling after myocardial infarction in rats. PloS One. 12: 0177521

Naclerio, F., Larumbe-Zabala, E., Cooper, R., Allgrove, J. and Earnest, C. P. (2015) A multi-ingredient containing carbohydrate, proteins L-glutamine and L-carnitine attenuates fatigue perception with no effect on performance, muscle damage or immunity in soccer players. PloS one. 10: 0125188

Neufcourt, L., Assmann, K., Fezeu, L., Touvier, M., Graffouillere, L., Shivappa, N., Hebert, J., Wirth, M., Hercberg, S., Galan, P., Julia, C and Kesse-Guyot, E. (2016) Prospective Association Between the Dietary Inflammatory Index and Cardiovascular Diseases in the SUpplementation en VItamines et Mineraux AntioXydants (SU.VI.MAX) Cohort. J. Am. Heart Assoc. 5: 002735

Ochoa, S. (1955) Malic dehydrogenase and 'malic' enzyme. In: Coloric, S.P., Kaplan, N. (Eds.), Methods of Enzymology, vol. I. Academic Press, New York, pp 735-745

Prenner, S. B., Mulvey, C. K., Ferguson, J. F., Rickels, M. R., Bhatt, A. B. and Reilly, M. P. (2014) Very low density lipoprotein cholesterol associates with coronary artery calcification in type 2 diabetes beyond circulating levels of triglycerides. Atherosclerosis. 236: 244-250

Prince, P. S. M., Rajakumar, S. and Dhanasekar, K. (2011) Protective effects of vanillic acid on electrocardiogram, lipid peroxidation, antioxidants, proinflammatory markers and histopathology in isoproterenol induced cardiotoxic rats. Eur. J. Pharmacol. 668: 233-240

Priscilla, D. H. and Prince, P. S. M. (2009) Cardioprotective effect of gallic acid on cardiac troponin-T, cardiac marker enzymes, lipid peroxidation products and antioxidants in experimentally induced myocardial infarction in Wistar rats. Chem. Boil. Interact. 179: 118-124

Qujeq, D., Omran, T. S. and Hosini, L. (2001) Correlation between total homocysteine, low-density lipoprotein cholesterol and high-density lipoprotein cholesterol in the serum of patients with myocardial infarction. Clin. Biochem. 34: 97-101

Rai, R. P. and Maurya, M. S. (1966) Synthesis and evaluation of antibacterial activity of vanillin derivatives. J Sci. Technol. India. 4: 275-276

Riccioni, G., Bucciarelli, T., Mancini, B., Corradi, F., Di Ilio, C., Mattei, P.A. and D'Orazio, N. (2007) Antioxidant vitamin supplementation in cardiovascular diseases. Ann. Clin. Lab. Sci. 37: 89-95

Salter, A. M. and White, D. A. (1996) Effects of dietary fat on cholesterol metabolism: regulation of plasma LDL concentrations. Nutr. Res. Rev. 9: 241-257

Shiny, K. S., Kumar, S. H., Farvin, K. H., Anandan, R. and Devadasan, K. (2005) Protective effect of taurine on myocardial antioxidant status in isoprenaline-induced myocardial infarction in rats. J. Pharm. Pharmacol. 57: 1313-1317

Stanely Mainzen Prince, P., Dey, P. and Roy, S. J. (2020) Sinapic acid safeguards cardiac mitochondria from damage in isoproterenol induced myocardial infarcted rats. J. Biochem. Mol. Toxicol. 34: 22556

Tejpal, C. S., Chatterjee, N. S., Elavarasan, K., Lekshmi, R. G. K., Anandan, R., Asha, K. K., Ganesan, B., Mathew, S. and Ravishankar, C.N. (2017) Dietary supplementation of thiamine and pyridoxine-loaded vanillic acid-grafted chitosan microspheres enhances growth performance, metabolic and immune responses in experimental rats. Int. J. Biol. Macromol. 104: 1874-1881

Thankappan, K. R., Shah, B., Mathur, P., Sarma, P. S., Srinivas, G., Mini, G. K., Daivadanam, M., Soman, B. and Vasan, R. S. (2010) Risk factor profile for chronic non-communicable diseases: results of a community-based study in Kerala, India. Indian. J. Med. Res. 131: 53-63

Tsuda, H., Uehara, N., Iwahori, Y., Asamoto, M., Ligo, M., Nagao, M., Matsumoto, K., Ito, M. and Hirono, I. (1994) Chemopreventive Effects of β Carotene, α Tocopherol and Five Naturally Occurring Antioxidants on Initiation of Hepatocarcinogenesis by 2 Amino 3 methylimidazo [4, 5 f] qumoline in the Rat. Jpn. J. Cancer Res. 85: 1214-1219

Upaganlawar, A., Gandhi, H. and Balaraman, R. (2010) Effect of vitamin E alone and in combination with lycopene on biochemical and histopathological alterations in isoproterenol-induced myocardial infarction in rats. J. Pharmacol. Pharmacother. 1: 24

Varma, R. S., Shukla, A. M. I. T. A. and Chatterjee, R. K. (1993) Evaluation of vanillic acid analogues as a new class of antifilarial agents. Indian J. Exp. Biol. 31: 819-821

Vishnu, K. V., Kumar, K. A., Chatterjee, N. S., Lekshmi, R. G. K., Sreerekha, P. R., Mathew, S. and Ravishankar, C. N. (2018) Sardine oil loaded vanillic acid grafted chitosan microparticles, a new functional food ingredient: attenuates myocardial oxidative stress and apoptosis in cardiomyoblast cell lines (H9c2). Cell Stress and Chaperones. 23: 213-222

Wooten, I. D. P. (1964) Microanalysis. *In*: *Medical Biochemistry* (ed. Churchill, J. and Churchill, A.), 4th edn., London. 101-107

Wroblewski, F. and Ladue, J. S. (1955) Lactic dehydrogenase activity in blood. Exp. Biol. Med. 90: 210-213

Yao, L., Liu, Z., Zhu, J., Li, B., Chai, C. and Tian, Y. (2016) Higher serum level of myoglobin could predict more severity and poor outcome for patients with sepsis. Am. J. Emerg. Med. 34: 948-952

Zheng, J., Huang, T., Yu, Y., Hu, X., Yang, B. and Li, D. (2012) Fish consumption and CHD mortality: an updated meta-analysis of seventeen cohort studies. Public Health Nutr. 15: 725-737

Zino, S., Skeaff, M., Williams, S. and Mann, J. (1997) Randomised controlled trial of effect of fruit and vegetable consumption on plasma concentrations of lipids and antioxidants. Bmj. 314: 1787