Canadian Journal of Physiology and Pharmacology

Systemic study of selected HDAC inhibitors in cardiac complications associated with cancer cachexia

Journal:	<i>Canadian Journal of Physiology and Pharmacology</i>
Manuscript ID	cjpp-2021-0012.R2
Manuscript Type:	Article
Date Submitted by the Author:	15-Jul-2021
Complete List of Authors:	Bora, Vivek; Nirma University, Department of Pharmacology Patel, Dhvani; Nirma University, Department of Pharmacology Johar, Kaid; Gujarat University, Department of Zoology, BMTC GOYAL, Ramesh; Delhi Pharmaceutical Sciences and Research University, Department of Pharmacology Patel, Bhoomika; Nirma University, Department of Pharmacology
Is the invited manuscript for consideration in a Special Issue:	IACS India 2019
Keyword:	histone deacetylase inhibitors, entinostat, MC1568, sodium butyrate, cardiac atrophy

SCHOLARONE™
Manuscripts

Systemic study of selected HDAC inhibitors in cardiac complications associated with cancer cachexia

Vivek Bora¹, Dhwani Patel¹, Kaid Johar², Ramesh K. Goyal³, Bhoomika M. Patel^{1*}

¹Department of Pharmacology, Institute of Pharmacy, Nirma University, Sarkhej-Gandhinagar Highway, Ahmedabad, Gujarat 382481

²Department of Zoology, BMTC, Human Genetics, USSC, Gujarat University, Ahmedabad, Gujarat, India

³Delhi Pharmaceutical Sciences Research University, Delhi, India

Draft

*Corresponding author

Dr. Bhoomika M. Patel

Assistant Professor

Institute of Pharmacy, Nirma University

Sarkhej- Gandhinagar Highway,

Ahmedabad 382 481, Gujarat, India

Phone No: + 91 79 30642718

Fax No: + 91 2717 241916

Email: drbhoomikampatel@gmail.com, bgoyal@nirmauni.ac.in

Abstract

Cancer cachexia is mainly characterized by wasting of skeletal muscles, fat and body weight loss, along with severe complications of major organs like liver, heart, brain and bone. There can be diminishing performance of these major organs as cancer cachexia progresses, one such drastic effect on the cardiac system. In present study, differential effect of histone deacetylase inhibitors (HDACi) on cardiac complications associated with cancer cachexia is studied. Two models were used to induce cancer cachexia: B16F1 induced metastatic cancer cachexia and LLC induced cancer cachexia. Potential of Class I HDAC inhibitor Entinostat, Class II HDAC inhibitor MC1568 and non-specific HDAC inhibitor sodium butyrate on cardiac complications were evaluated using the cardiac hypertrophy markers, hemodynamic markers and cardiac markers along with histopathological evaluation of heart sections by periodic acid schiff staining, masson trichrome staining, picro sirius red staining and haematoxylin and eosin staining. Immunohistochemistry evaluation by vimentin and caspase 3 protein expression was evaluated. Entinostat showed promising results by attenuating the cardiac complications, MC1568 treatment further exacerbated the cardiac complications while non-conclusive effect were recorded after treatment with sodium butyrate. Further, this study will be helpful in evaluating other HDAC inhibitors for potential in cardiac complications associated with cancer cachexia.

Key words: Cardiac atrophy, histone deacetylase, histone deacetylase inhibitors, entinostat, MC1568, sodium butyrate.

Introduction:

Cancer cachexia (CC) is a major factor for cancer associated deaths worldwide. Worldwide cancer cachexia is the cause of 20-30% of deaths due to cancer (Fearon et al. 2011). About 80% of cancer patients suffer from cancer cachexia and the burden of cancer cachexia is predicted to increase worldwide in near future (Lim et al. 2020). Cancer cachexia has prominent characteristics as loss of body weight, loss of appetite or anorexia, systemic inflammation, wasting of fat mass and muscle mass and also impairment in major metabolic processes like carbohydrate and protein metabolism (Patel et al. 2017; Dave et al. 2019; Lim et al. 2020). Moreover, cancer cachexia is also associated with alternations in cardiac architecture and hemodynamics (Patel and Damle 2013; Sukumaran et al. 2016). Cancer cachexia induced atrophy of heart occurs by activating the signalling pathway which governs the protein synthesis and degradation. In cancer cachexia patients, cardiac protein degradation signalling pathways get activated and the cardiac protein synthesis pathway get inhibited causing overall protein degeneration and atrophy of heart (Stevens et al. 2015)

Histone deacetylases (HDACs) are the major enzymes involved in the process of acetylation and deacetylation of deoxyribonucleic acid (DNA). Thus, expression patterns of different cells present in the body are regulated by these group of enzymes called as HDACs. In normal conditions one or many HDACs are present in the different tissues responsible for their specific function and their functional activity is dependent on the activity of respective HDACs (De Ruijter et al. 2003). The HDACs are also present in cancer tissues and their expression is altered in cancer (Olzscha et al. 2015; De Ruijter et al. 2003). The role of HDACs in cancer is vital as they are involved in processes like alteration in cancer cell proliferation and differentiation, cytostasis, angiogenesis, immune responses, apoptosis and necrosis (De Ruijter et al. 2003; Olzscha et al. 2015).

The role of HDAC in heart is varying and has been shown to control events including hypertrophy, fibrosis, contractility, autophagy and energy metabolism (Patel 2019). Various

data suggest that class I and class II HDACs play opposing roles in the regulation of hypertrophic pathways. A study reported the functional role of HDACs in regulation of cardiovascular functions like growth of myocardium, morphogenesis and contractility of myocardium, the cardiac functions were improved significantly in HDAC knockout mice (Montgomery et al. 2007). Kee et al. 2006 reported predominant involvement of class I HDACs in hypertrophic response on aortic banding or angiotensin II infusion induced hypertrophy animal models. However, among HDAC IIa and IIb HDAC2, 4, 5, and 9 have been reported to be involved in hypertrophic responses; HDAC4, 5, and 9 are negative regulators (Eom et al. 2015). Nonspecific HDAC produces beneficial effect on cardiac hypertrophy. The beneficial effect of sodium butyrate is arbitrated through downregulation of class I HDACs, specifically HDAC2 without any effect on class II HDAC, i.e., HDAC5 (Patel 2018). Different classes of HDAC exert differential cardiac effects and hence, we speculate that different classes of HDAC inhibitors (HDACi) may have different effects in cancer cachexia. Hence, we have selected entinostat (MS275) selective as class I HDAC inhibitor, MC1568 selective class II HDAC inhibitor and sodium butyrate as non-specific HDACs inhibitor to study their effects in cancer cachexia.

Entinostat is a synthetic benzamide derivative, It is a potent and selective class I HDACs inhibitor with a long half-life (relatively between 33 and 52 hours) (Ryan et al. 2005) specially inhibiting HDAC1,2, 3 (Knipstein et al. 2011) with IC_{50} of 0.51 μ M and 1.7 μ M respectively (Souza et al. 2015; Leus et al. 2017). MC 1568 is a selective class II (IIA) histone deacetylase inhibitors (HDAC4 and HDAC 5) with IC_{50} of 220nM (Usui et al. 2014). Sodium butyrate is the sodium salt of butyric acid. Butyric acid is a short chain fatty acid produced by bacterial fermentation of dietary fibre within the gastrointestinal tract. It is actively absorbed by intestinal epithelial cells and is the major luminal source of energy for colonocytes (Reddy 1999; Bennett et al. 1996). Its anticancer activity is ascribed to its non- specific HDACs inhibitor action, inhibiting both class I and class II HDAC, mostly on HDAC2 and HDAC5

(De Ruijter et al. 2003; Patel 2018). Despite varied reports of entinostat (MS275), MC1568 and sodium butyrate on cancer, no reports are available with respect to effect of these agents in cancer cachexia. Hence, we thought it worthwhile to evaluate the effect of different classes of HDACs inhibitors on cardiovascular complication associated with cancer cachexia.

Material and Methods:

Drugs, antibodies and chemicals

Chemicals used in the study were of analytical grade. Phosphotungstic acid, Phosphomolybdic acid, Aniline Blue and Haematoxylin working solution was purchased from SRL Pvt. Ltd. Direct 80 and Biebrich Scarlet were brought from Sigma Aldrich. Test drugs Entinostat and MC1568 were brought from ADOOQ Bioscience LLC, while gift sample of Sodium butyrate was received from Vaibhav Industries 216, Jhalawar, Patanwal Complex, Lbs Marg, Ghatkopar (W), Mumbai, India.

Cell culture

B16F1 melanoma cell line was procured from National Centre for Cell Science, Pune, India and Lewis lung carcinoma cells LL/2 (LLC1) (ATCC® CRL-1642™) was purchased from American Type Culture Collection, Manassas, VA, USA. For both cell lines, complete growth medium was used for culturing. Complete growth media consisted of Dulbecco's modified Eagle medium (DMEM) with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37°C and 5% CO₂.

Animal models

Institutional animal ethical committee (IAEC), Institute of Pharmacy, Nirma University, Ahmedabad approved the experimental protocol according to the guidelines issued by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Fisheries, Animal Husbandry and Dairying Department of Animal Husbandry and Dairying, Government of India. Animals were treated in accordance with Guide for the Care and Use of Laboratory Animals (8th edition, National Academies Press).

Mice of Balb/c or C57BL/6 strain of either sex, weighing between 35-40 gm of 10-12 weeks were used for the experiment. During the experimental study mice were given free access to conventional dietary feed and water *ad libitum*.

Five experimental groups were designed and the mice Balb/c or C57BL/6 were grouped respectively. The experimental groups were as 1) Normal control mice (NC mice) receiving phosphate buffer saline (PBS); 2) Cancer cachexia animals (disease control) (DC mice) injected with B16-F1 or LLC cells; and 3) Cancer cachexia animals injected with B16-F1 or LLC cells treated with Entinostat (20mg/kg/p.o.) (EN mice). 4) Cancer cachexia animals injected with B16-F1 or LLC cells treated with MC1568 (50mg/kg/i.p.) (MC mice). 5) Cancer cachexia animals injected with B16-F1 or LLC cells treated with Sodium butyrate (60mg/kg/i.p.) (SB mice). Experimental drugs were reconstituted as follows, for entinostat, 30% propylene glycol, 5% tween 80, 65% of 5% dextrose in water, final concentration was set at 20mg/kg was used and oral route was followed for administration to mice in respective group. For MC1568, 0.5% carboxy methyl cellulose (CMC) solution was made, final concentration was set to 50mg/kg and intra peritoneal route was followed for administration to mice in respective group. For sodium butyrate, distil water was used and final concentration was set to 60mg/kg and oral route was followed for administration to mice in respective group. All the treatment groups received respective doses once in a day.

B16F1 induced metastatic cancer cachexia

After culturing the B16F1 cells, the cells were injected into Balb/c mice at final concentration of 1×10^6 cells/mL/mice. Phosphate buffer saline (PBS) was used to resuspend the cells to final concentration and inject in respective group mice. Sterilized PBS was injected in the normal control mice. To inject the cells, a rapid (within 5 sec) large volume (7.5% of body weight) hydrodynamic injection was given by tail vein route. Followed by induction period of 3 weeks was allowed for development of metastatic cancer (Li et al. 2011). Post induction period treatment of respective HDAC inhibitors was given once in a day for two weeks. Later, for

biochemical parameter examination blood was collected followed by recording of hypertrophic and hemodynamic parameters.

LLC (Lewis lung carcinoma cells) induced cancer cachexia

After culturing the LLC cells, the cells were injected into the right flank region of the C54BL/6 mice by subcutaneous injection. Prior to injection the right flank of the mice was shaved. The LLC cell inoculum consisting cell concentration at 5×10^7 cells/mL was used, the cells were resuspended in 100 μ L phosphate buffer saline (PBS). In normal control mice 100 μ L of PBS was injected, respectively. 21 days induction period was given for tumor growth (Sun et al. 2016). Post induction period treatment of respective HDAC inhibitors was given for two weeks. Later, for biochemical parameter examination blood was collected followed by recording of hypertrophic and hemodynamic parameters.

Blood sample collection, serum analysis

Retro orbital plexus route was used for collection of blood. Under light ether anesthesia blood was collected in centrifuge tubes. Blood was kept at room temperature for 30 min to allow clotting. After 30 min the serum was collected by centrifuging for 20 min at 4000 rpm. Serum was stored at -20°C and was used for biochemical parameter estimation. Lactate dehydrogenase (LDH) and Creatinine Kinase (CK-MB), Troponin I in serum were estimated by using biochemical diagnostic kits (Labcare Diagnostics Pvt. Ltd., India) through biochemical analyzer (Prietest TOUCH Biochemistry Analyzer, 2.622 A, ROBONIK Pvt. Ltd.) (Goyal et al. 2008; Goyal et al. 2009; Goyal et al. 2011; Patel et al. 2013).

Hemodynamic estimation

For estimation of hemodynamic parameters transducer (BP 100) and LabScribe Systems (IWORX, New Hampshire, USA) were used. Mice were anesthetized using Ketamine (20 mg/kg, i.p.) + Xylazine (10 mg/kg, i.m.). Throughout the experiment, body temperature was maintained at $37 \pm 1^{\circ}\text{C}$. Hemodynamic parameters were measured by cannulating the carotid artery. Mean blood pressure (MABP), heart rate, rate of pressure development

($dp/dt_{\max}$) and rate of pressure decay ($dp/dt_{\min}$) were measured. LabScribe software (version 118) was used for analysis of data (Raghunathan et al. 2014; Patel & Bhadada 2014; Rayabarapu et al. 2014)

Hypertrophic estimation

Mice were sacrificed after haemodynamic parameter estimation and the heart was isolated. After isolation the heart was blotted with blotting paper and any traces of blood was removed. Weight of heart was recorded, cardiac hypertrophic index (wet heart weight to femur length) was calculated, left ventricular (LV) and right ventricular (RV) weights were recorded (Patel & Desai 2014; Patel et al. 2012; Goyal et al. 2012)

Tissue collection

Heart tissue was isolated in both models and collagen content (Sharma et al. 2019) and $Na^+K^+ATPase$ (Patel, Raghunathan, et al. 2014; Rabadiya et al. 2018) activity in heart tissue was estimated as reported earlier.

Protein expression

In LLC induced cancer cachexia model, protein estimation of caspase-3 was done. After estimation of total protein in the heart tissue homogenate extracted by using 5X Sample buffer (loading buffer): 10% w/v SDS 10 mM Dithiothreitol, or beta-mercapto-ethanol 20%v/v Glycerol 0.2M Tris-HCl, pH 6.8 0.05% w/v Bromophenolblue. The target protein was dissolved in the liquid phase with freshly added 1% protease inhibitor cocktail and 1 mM phenylmethylsulphonyl fluoride (PMSF). 80µg of protein was subjected to a 10% SDS-PAGE gel to separate the proteins by gel electrophoresis and then transferred onto polyvinylidene fluoride (PVDF) (0.45 mm; Millipore, Boston, MA) membranes. Membranes were blocked for 1 h at 37°C in 5% (w/v) nonfat dried skim milk (blocking buffer), followed by incubation overnight at 4°C with primary antibodies in blocking buffer. Post incubation, washing of primary antibody was done, and the membrane was incubated with secondary antibody for 1hr in blocking buffer. After incubation with secondary antibody, the membrane was washed and

electrochemiluminescence (ECL) reagents (Thermo Fisher Scientific) were used for detection of proteins and visualized using the GelDoc XR Gel Documentation System (Bio-Rad, San Francisco, CA). Image J (version 1.52h) software was used for quantitative analyses of protein expression.

Histopathological studies

Haematoxylin and Eosin (HE) staining was used to determine atrophy of heart. Later, tissue was stained with masson trichrome for observing connective tissues and picro-sirius red staining was performed for observing collagen I and collagen III in the heart tissue. Periodic acid schiff stain was performed to observe the glycoprotein, glycogen, mucin and glycolipids levels in the heart tissue. Using a digital camera (Olympus EP50 camera) images were acquired after staining.

Immunohistochemistry staining of heart tissue in B16F1 induced cancer cachexia model by vimentin was done. Vimentin is a marker for atrophy in cardiac tissue. After staining the images were captured using digital camera (Olympus EP50 camera).

Statistical analysis

For statistical analysis comparison between normal control and disease control was performed and between disease control and disease treated groups was done. One- way ANOVA followed by Tukey's posttest was applied. All the values are expressed as mean $\pm$ S.E.M. For statistical analysis, Prism 5.01 (GraphPad Software) was used. Statistical significant results have differences of $p < 0.05$.

Results:

Effect of HDAC inhibitors on hypertrophy markers

Effect of HDAC inhibitors in both models was studied by hypertrophy markers as cardiac hypertrophy index, weight of right and left ventricle and weight of heart. In both models there was significant ($p < 0.05$) decrease in cardiac hypertrophy index in disease control group and MC1568 treated group when compared with normal group. In B16F1 model, treatment with

entinostat and sodium butyrate showed significant ($p<0.05$) increase in cardiac hypertrophic index while treatment with MC1568 did not show significant ($p<0.05$) increase in cardiac hypertrophy index when compared with disease control group. In LLC model, treatment with entinostat showed significant ($p<0.05$) increase in cardiac hypertrophic index while treatment with MC1568 and sodium butyrate did not show significant ($p<0.05$) increase in cardiac hypertrophy index when compared with disease control group (Figure 1(A.i), Figure 1(B.i). In both models, there was significant ($p<0.05$) decrease in right ventricle (RV) weight, left ventricle (LV) weight and heart weight in disease control group, MC1568 treated group and sodium butyrate treated group when compared with normal control group. In LLC models, there was significant ($p<0.05$) decrease in right ventricle (RV) weight, left ventricle (LV) weight and heart weight in entinostat treated group when compared with normal control group, and significant ($p<0.05$) decrease in left ventricle weight was observed in B16F1 model when compared with normal control group. Treatment with entinostat showed significant ($p<0.05$) increase in right ventricle (RV) weight, left ventricle (LV) weight and heart weight, while treatment with MC1568 and sodium butyrate did not show significant ($p<0.05$) increase in right ventricle (RV) weight, left ventricle (LV) weight and heart weight when compared with disease control group (Figure 1(A.ii, A.iii, A.iv), Figure 1(B.ii, B.iii, B.iv).

Effect of HDAC inhibitors on cardiac markers

Effect of HDAC inhibitors in both models was studied by cardiac markers such as collagen in heart tissue, serum creatinine kinase (CK-MB), serum lactate dehydrogenase (LDH), $\text{Na}^+\text{K}^+\text{ATPase}$ activity in heart tissue and serum Troponin I. Collagen content in heart tissue was decreased significantly ($p<0.05$) in disease control group and MC1568 treated group in both models when compared with normal control groups. Collagen content in heart tissue was decreased significantly ($p<0.05$) in sodium butyrate group in B16F1 model when compared with normal control groups. In B16F1 model treatment with entinostat produced significant ($p<0.05$) increase in collagen content in heart tissue while treatment with MC1568 and sodium

261 butyrate produced no change in collagen content in heart tissue when compared with disease
262 control group. In LLC model treatment with entinostat and sodium butyrate showed significant
263 ($p<0.05$) increase in collagen content in heart tissue while treatment with MC1568 produced
264 no change in collagen content in heart tissue when compared with disease control group (Figure
265 2(A.i), Figure 2(B.i). In both models, serum creatinine kinase (CK-MB), serum lactate
266 dehydrogenase (LDH) and serum troponin I levels in disease control group and MC1568
267 treated group were significantly ($p<0.05$) increased when compared with normal group. In
268 B16F1 model, serum creatinine kinase (CK-MB) levels in entinostat treated group were
269 significantly ($p<0.05$) decreased when compared with normal group. In both models, serum
270 lactate dehydrogenase (LDH) levels were significantly ($p<0.05$) increased in sodium butyrate
271 treated group when compared with normal control group. In LLC model, serum lactate
272 dehydrogenase (LDH) levels were significantly ($p<0.05$) increased in entinostat treated group
273 when compared with normal control group. Treatment with entinostat and sodium butyrate
274 produced significantly ($p<0.05$) decreased levels of serum creatinine kinase (CK-MB), serum
275 lactate dehydrogenase (LDH) and serum troponin I, while no change was observed after
276 treatment with MC1568 in both models when compared to disease control group (Figure 2(A.ii,
277 A.iii, A.v), Figure 2(B.ii, B.iii, B.v). In both models, $\text{Na}^+\text{K}^+\text{ATPase}$ activity in heart tissue in
278 disease control group was significantly ($p<0.05$) decreased when compared with normal group.
279 Treatment with entinostat and sodium butyrate produced significantly ($p<0.05$) increased
280 $\text{Na}^+\text{K}^+\text{ATPase}$ activity in heart tissue, while no change was observed after treatment with
281 MC1568 in both models when compared to disease control group (Figure 2(A.iv), Figure
282 2(B.iv).

283 *Effect of HDAC inhibitors on hemodynamic markers*

284 Effect of HDAC inhibitors in both models was studied by recording hemodynamic markers
285 like, mean blood pressure, systolic pressure, diastolic pressure, heart rate, rate of pressure
286 development and rate of pressure decay. In B16F1 model and LLC model, mean blood

pressure, systolic pressure and diastolic pressure in disease control group were significantly ($p < 0.05$) increased when compared with normal group. In both models, mean blood pressure and systolic pressure in MC1568 treated group were significantly ($p < 0.05$) increased when compared with normal group. Treatment with entinostat and sodium butyrate produced significantly ($p < 0.05$) decreased mean blood pressure, systolic pressure and diastolic pressure, while no change was observed after treatment with MC1568 in both models when compared to disease control group (Figure 3(A.i, A.ii, A.iii), Figure 3(B.i, B.ii, B.iii)). In both models, heart rate in disease control group was significantly ($p < 0.05$) increased when compared with normal group. Treatment with entinostat and sodium butyrate produced significant ($p < 0.05$) decrease in heart rate in both models when compared to disease control group. Treatment with MC1568 in B16F1 model produced no change in heart rate, but treatment with MC1568 in LLC model produced significant ($p < 0.05$) decrease in heart rate when compared with disease control group (Figure 3(A.iv), Figure 3(B.iv)). In both models, rate of pressure development and rate of pressure decay in disease control group and MC1568 treated group was significantly ($p < 0.05$) decreased when compared with normal group. In LLC model, rate of pressure development in entinostat treated group was significantly ($p < 0.05$) decreased when compared with normal group. In both models, rate of pressure decay in entinostat treated group and sodium butyrate treated group was significantly ($p < 0.05$) decreased when compared with normal group. Treatment with entinostat and sodium butyrate produced significant ($p < 0.05$) increase in rate of pressure development and rate of pressure decay when compared to disease control group. Treatment with MC1568 in B16F1 model produced no change in rate of pressure development and rate of pressure decay when compared to disease control group. Treatment with MC1568 in LLC model produced significant ($p < 0.05$) increase in rate of pressure development but no change was observed in rate of pressure decay when compared with disease control group (Figure 3(A.v, A.vi), Figure 3(B.v, B.vi)).

Caspase 3 is an important apoptosis marker. In B16F1 model, protein expression of caspase-3 was found to be significantly ($p<0.05$) increased in the disease control group when compared to normal control group. Treatment with entinostat, MC1568, sodium butyrate showed significantly ($p<0.05$) decreased expression of caspase-3 when compared with disease control group (Figure 5(E)).

Discussion:

Many disease conditions like cancer, cancer cachexia may lead to heart related diseases and thus may lead to cardiac hypertrophy. Cardiac hypertrophy can lead to heart failure due to cardiac dysfunction (Kook et al. 2011). HDAC play a vital role in cardiac hypertrophy. Expression of Class II HDACs is widely found in heart while expression of Class I HDACs is ubiquitous and HDAC2 is highly expressed in heart. HDAC1 is involved in the regulation of cardiomyogenesis, embryonic development and cell proliferation (Liu et al. 2009). Main reason for cardiac hypertrophy activity of HDAC2 is the elevation of functional activity of HDAC2 enzyme in response to hypertrophic stimuli regulated by posttranslational modification (Kee et al. 2008). Role of Class II HDACs like HDAC5 and HDAC9 in cardiac hypertrophy are explored till now, HDAC9 acts as negative regulator of cardiac hypertrophy in deacetylase enzyme-independent form as a transcriptional repressor (Eom et al. 2015). Distinctive to HDAC9, HDAC5 deacetylates the HDAC2 and attenuates hypertrophic action of HDAC2 (Eom et al. 2014; Eom et al. 2015).

The cancer cachexia progression results in left ventricular mass loss and further causes impaired cardiac function (Tian et al. 2011). Cachexia results in the myocardium wasting, giving rise in cardiac atrophy (Matsumoto et al. 1999). The cardiac hypertrophy index is defined as the ratio of wet heart weight to the femur length (Patel & Desai 2014). In present study investigation, B16F1 and LLC induced cancer cachexia model there was significant decrease cardiac hypertrophy index, left ventricular weight, right ventricular weight, heart weight in diseased control animals as compared to normal animals indicating cardiac atrophy.

Treatment with entinostat and sodium butyrate produced a marked increase in the cardiac hypertrophy index, left ventricular weight, right ventricular weight, heart weight as compared to diseases control animals, whereas MC1568 produce the decrease effect on cardiac hypertrophy index, left ventricular weight, right ventricular weight and heart weight. In LLC induced cancer cachexia model, entinostat and sodium butyrate produced a significant decrease in the cardiac hypertrophy index, left ventricular weight, right ventricular weight, heart weight as compared to diseases control animals whereas there was increase in MC1568 treated animals. It has been previously reported that HDAC2 causes cardiac hypertrophy and repression of proliferation, whereas HDAC3 act to inhibit cardiac hypertrophy with increasing proliferation (Kook et al. 2011). Since entinostat is act on HDAC 3, it increases cardiac hypertrophic index, right and left ventricular weight and heart weight due to inhibition of cardiac hypertrophic. Class I and II HDACs has been shown opposing role in regulation of cardiac myocyte growth and heart diseases. HDAC 2 class I HDAC, induces cardiac hypertrophy in response to stimuli of hypertrophic, whereas class II HDACs including HDAC 4, 5, and 9 increases the cardiac hypertrophic (Kook et al. 2011; Eom et al. 2015). Since MC1568 is specific class II HDAC decreases the cardiac hypertrophy associated with cancer cachexia.

Reduction in collagen levels in cancer cachexia indicates atrophy (Bora and Patel 2021). In present study, B16F1 and LLC induced cancer cachexia model there was significant decrease collagen level in diseased control animals as compared to normal animals. Treatment with entinostat produced a marked increase in the collagen level as compared to diseases control animals, whereas MC1568 and sodium butyrate produced further decrease in collagen level. In LLC induced cancer cachexia model, entinostat and sodium butyrate produced a significant increase in the collagen level as compared to diseases control animals whereas there was no effect in MC1568 treated animals. Study reported that Trichostatin A (TSA) is an HDACi to blunt collagen biosynthesis in overload pressure induced cardiac hypertrophy mice

model, it also have antifibrogenetic action was pointed (Kong et al. 2006). Since entinostat is class I HDAC inhibitor increases the collagen level due to inhibition of cardiac hypertrophy (which is associated with increased collagen levels). Phenylbutyrate was found to increase collagen type 1 levels in lung fibroblast of human by its cyclic adenosine monophosphate (cAMP) regulatory mechanism and HDAC inhibitory action (Rishikof et al. 2004). Since sodium butyrate is nonspecific and it possibly increases collagen level by its action on cAMP regulatory mechanism.

Higher concentration of CK-MB have been found in heart muscle of animals and human myocardium with heart failure, aortic stenosis and coronary artery diseases (Ay et al. 2002). The coric cycle is most important for cachexia due to tumor induced lactic acid production which causes conversion from anaerobic to aerobic in tumor beacause of gluconeogenesis (Zhou 2005). The more energy required for generation of gluconeogenesis and conversion from anaerobic to aerobic energy production enzyme is responsible know as lactate dehydrogenase (Zhou 2005). In present study investigation, B16F1 and LLC induced cancer cachexia model there was significant increase CK-MB and LDH levels in diseased control animals as compared to normal animals. Treatment with entinostat and sodium butyrate produced a marked decreased in the CK-MB and LDH levels as compared to diseases control animals, whereas MC1568 produce the increase effect on CK-MB and LDH levels in both the models. Class I HDACi shows cardioprotective effects in nondiabetic heart I/R Injury by decreasing CK-MB and LDH and also activating PI3K/Akt signalling pathway, modulated mitochondrial apoptotic pathways via Foxo3a/Bim (Wu et al. 2017). Since entinostat is class I HDACi decreases CK-MB Level by activating PI3K/Akt signalling pathway. Study reported that Inhibition of HDAC class II activity exacerbates rather than attenuates CVB3-induced viral myocarditis in BALB/c mice and increases the CK-MB and LDH level by activation of pyrin domain containing 3 (NLRP3) and IL-1 β secretion in mediating the pathological response to CVB3 viral infection (Wang et al. 2014). Since MC1568 is class II HDACi increases the CK-MB level may be due

to activation of pyrin domain containing 3 (NLRP3) and IL-1 β secretion. Daosukho et al. 2007 reported significant reduction of CK activity- a non- specific but widely used cardiac injury markers on treatment with phenylbutyric acid, an HDACi.

Na⁺K⁺ATPase dysfunctioning has been found to be associated with myocardial contractile dysfunction (Horton et al. 2007). In present study, B16F1 and LLC induced cancer cachexia showed significant reduction in Na⁺K⁺ATPase activity as compare to normal control animals. In B16F1 and LLC cancer cachexia animals treated with entinostat and sodium butyrate produced significant increase in Na⁺K⁺ATPase activity as compare with disease control animals, whereas MC1568 treatment didn't produce any effect in both the models. It has been reported that Na⁺K⁺ATPase activity during diseased conditions is regulated by PKC activity (Horton et al. 2007; Fedorova et al. 2003). Study reported that HDAC2 increases the PKC activation in rat cardiomyocytes (Li et al. 2015). Since entinostat is HDAC class I inhibitor, it increases the Na⁺K⁺ATPase activity by decreasing the Protein kinase C (PKC) activity. Since MC1568 is Class II HDACi and increases Na⁺K⁺ATPase activity in LLC model may be due to reverse effect of sodium influx and interaction between epidermal growth factor (EGF) and tubulin (Li et al. 2015; Li et al. 2016). Valproic acid has been reported to reduce PKC expression and levels of membrane associated PKC in cultured glioma cells and rat brain (Hahn et al. 2005). Since sodium butyrate is nonspecific HDACi restored the Na⁺K⁺ATPase activity by reducing PKC activation.

Troponin I is a thin filament inhibitory complex that inhibits actin-myosin activity at the diastolic levels of the intracellular calcium. In present study, B16F1 and LLC induced cancer cachexia model there was significant increase troponin-I level in diseased control animals as compared to normal animals. Treatment with entinostat and sodium butyrate produced a marked decrease in the troponin-I level as compared to diseases control animals, whereas MC1568 produced no change in troponin-I level in both the models. Class I HDACs are reported to actively repress the expression of *CACNA2D2* during normal cardiac growth and

416 increases troponin-I level (Montgomery et al. 2007). Since entinostat is class I HDACi and
417 decreases troponin-I level might be due to downregulation of T and L type of calcium channel.
418 Class II HDAC enzymes have been implicated as inhibitors of pro-hypertrophic pathways.
419 HDAC 4, 5 and 9 have been found to interact with myocyte enhancer factor- 2 (MEF2) and
420 regress hypertrophy causes decrease in troponin-I (Chang et al. 2004). Since MC1568 is class
421 II HDACi and it did not decreases troponin-I may be due to interaction of HDAC with MEF2.
422 Since sodium butyrate is nonspecific class I/II HDACi and have more potent action on HDAC2
423 decreases the troponin-I level might be due to downregulation of T and L type of calcium
424 channel.

425 The elevation in the cytokine levels in cachexia causes the stimulation of NF-kB signalling,
426 which is the mediator of muscle loss and produces significant cardiac dysfunctions (Wysong
427 et al. 2011). In present study, B16F1 and LLC model there was significant increase in blood
428 pressure, heart rate, systolic and diastolic pressure and significant decrease in rate of pressure
429 decay and development in disease control animals as compare to normal control animals. In
430 both B16F1 and LLC models, treatment with entinostat and sodium butyrate produced decrease
431 in blood pressure, heart rate, and significant increase in rate of pressure decay and development,
432 whereas MC1568 did not produced any marked effect. Magnesium valproate is reported to
433 significantly improve the hemodynamics by reducing blood pressure, heart rate and restoring
434 left ventricular functioning due to HDAC2 inhibition (Raghunathan et al. 2016). Study reported
435 that inhibitors of HDAC5 produced no significant changes in heart rate or systemic blood
436 pressure (Zhao et al. 2012). Since MC1568 is class II HDACi and has no effect on blood
437 pressure and heart rate and marked decrease in mean pressure may be due to decrease in
438 monocyte chemoattractant protein-1 (MCP-1) and platelet-derived growth factor (PDGF).

439 Pathways to caspase-3 activation have been identified that are either dependent on or
440 independent of mitochondrial cytochrome c release and caspase-9 function (López-Otín et al.
441 2007). In present study, cancer cachexia animals exhibited increased protein level of caspase 3

in heart as compare to normal control animals suggesting increased apoptosis. Treatment with entinostat and sodium butyrate produced marked decreases in protein level of caspase 3 which shows the reverse condition from cell death as compare with disease control animals; whereas MC1568 did not show marked decrease in caspase 3 level as compared with disease control animals. Induction of autophagic cell death by HDAC class I inhibitors has clear clinical implications in treating cancers with apoptotic defects (Zhang et al. 2014). Since entinostat is class I HDAC inhibitor, it decreases protein expression in skeletal muscle and help in reversing the condition of cancer cachexia.

Periodic acid Schiff (PAS) is a staining represent that glycogen was abnormally distributed in the interstitial space as compare with normal control animals (Nojiri et al. 2006). In current investigation, cancer cachexia animals exhibited heart with enlarged cardiomyocytes, increase fibrosis and decrease storage of glycogen and mucin, with abnormally distribution of the interstitial spaces. Treatment with entinostat and sodium butyrate produced increase in accumulation of glycogen level and showed beneficial effect in cancer cachexia as compare to normal control animals, whereas MC1568 produced decrease in accumulation of glycogen (Figure 4(A), Figure 5(A). Masson trichrome stain (MT) is used to differentiate between collagen and smooth muscle in tumours. A study demonstrated masson trichrome to identify the fibrosis of LV myocardium in cardiac atrophy (Tsai et al. 2012). In present study, in cancer cachexia animals there was significant increase in atrophy of connective tissues of heart, increase in interstitial spaces and decrease in collagen level as compared to normal control animals. Treatments with entinostat and sodium butyrate produced decrease in atrophy of connective tissue, decreased interstitial spaces and increase in collagen level, whereas MC1568 produce atrophy of connective tissues, increase in interstitial spaces and decrease in collagen level as compared to disease control animals (Figure 4(B), Figure 5(B). Picro Sirius red (SE) stain is used to stain the collagen I and III present in muscle fibre. Study reported in which picro Sirius red stain was used to identify the fibrosis of LV

myocardium and differentiate collagen I and III in cardiac atrophy (Tsai et al. 2012). In present study, there was significant increase in atrophy of connective tissues, increase in interstitial spaces and decrease in collagen level as compared to normal control animals. Treatments with entinostat and sodium butyrate produced decrease in atrophy of connective tissue, decreased interstitial spaces and increase in collagen level, whereas MC1568 produce atrophy of connective tissues, increase in interstitial spaces and decrease in collagen level as compared to disease control animals (Figure 4(C), Figure 5(C). Haematoxylin and eosin (H&E) stain was used to study the atrophy in heart (Cosper et al. 2011). In disease control group there was decrease in mass of heart and increase in inflammation when compared to normal control group. The loss of mass was confirmed the atrophy of heart while treatment with entinostat and sodium butyrate attenuated the atrophy of heart while MC1568 treatment failed to prevent atrophy of heart when compared to disease control group (Figure 4(D), Figure 5(D).

Vimentin is a structural protein that in humans is encoded by the VIM gene. During cardiac atrophy vimentin protein level gets decreases (Camelliti et al. 2005). It has been reported that HDAC1 and HDAC2 displayed differential expression patterns where HDAC1 mainly expressed in cardiac fibroblast and HDAC2 in cardiomyocytes, and upregulation of HDAC1 and 2 in congestive heart failure (CHF). In addition, HDAC inhibition reverses interstitial fibrosis in CHF. Possible anti-fibrotic actions of HDAC inhibition include reversal of myofibroblast activation, increase vimentin level and induction of cell cycle arrest/apoptosis (Nural-Guvener et al. 2014). Since entinostat is class I HDACi, it reverses the interstitial fibrosis and increases the vimentin in cardiac fibroblast. Study reported that Trichostatin A (TSA) or valproic acid (VPA) induced mesenchymal features in the colon carcinoma cells by a decrease in E-cadherin and an increase in vimentin expression at the mRNA and protein levels (Ji et al. 2015). Since sodium butyrate is nonspecific HDACi and increases the vimentin in cardiac fibroblast and prevent the cardiac atrophy condition (Figure 4(E).

Conclusion:

In present study cardiac complications associated with cancer cachexia were studied. The class I HDAC inhibitors showed promising results on cardiac complications associated with cancer cachexia. Deleterious effects on cardiac complications were observed after treatment with class II HDAC inhibitor. Non-specific HDAC inhibitor showed non-conclusive effects on cardiac associated complications in cardiac cachexia. The data generated will be helpful in designing further studies for management of cardiac complications associated with cardiac cachexia.

Competing interests:

The authors declare there are no competing interests.

Funding:

This research was supported by Science and Engineering Research Board, Department of Science and Technology, Government of India (Grant no. SB/YS/LS-144/2014).

References:

- Ay, H., Arsava, E.M., & Sarba, O. 2002. Creatine kinase-MB elevation after stroke is not cardiac in origin comparison with troponin T levels. *Stroke* 33(1): p.286–289. Available at: <https://pubmed.ncbi.nlm.nih.gov/11779925/> [Accessed December 30, 2020].
- Bennett, W.G., & Cerda, J.J. 1996. Benefits of dietary fiber: Myth or medicine? *Postgraduate Medicine* 99(2): p.153–166. Available at: <https://europepmc.org/article/med/8632964> [Accessed December 30, 2020].
- Bora V, Patel BM. Investigation into the role of anti-diabetic agents in cachexia associated with metastatic cancer. *Life Sciences* June 2021, 274: 119329
- Camelliti, P., Borg, T.K., & Kohl, P. 2005. Structural and functional characterisation of cardiac fibroblasts. *Cardiovascular Research* 65(1): p.40–51. Available at: <https://pubmed.ncbi.nlm.nih.gov/15621032/> [Accessed December 30, 2020].
- Chang, S., McKinsey, T.A., Zhang, C.L., Richardson, J.A., Hill, J.A., & Olson, E.N. 2004. Histone Deacetylases 5 and 9 Govern Responsiveness of the Heart to a Subset of Stress

- 520 Signals and Play Redundant Roles in Heart Development. *Molecular and Cellular*
 521 *Biology* 24(19): p.8467–8476. Available at: [/pmc/articles/PMC516756/?report=abstract](https://pubmed.ncbi.nlm.nih.gov/24516756/)
 522 [Accessed December 30, 2020].
- 523 Cosper, P.F., & Leinwand, L.A. 2011. Cancer causes cardiac atrophy and autophagy in a
 524 sexually dimorphic manner. *Cancer Research* 71(5): p.1710–1720. Available at:
 525 [/pmc/articles/PMC3049989/](https://pubmed.ncbi.nlm.nih.gov/213049989/) [Accessed May 6, 2021].
- 526 Daosukho, C., Chen, Y., Noel, T., Sompol, P., Nithipongvanitch, R., Velez, J.M., et al. 2007.
 527 Phenylbutyrate, a histone deacetylase inhibitor, protects against Adriamycin-induced
 528 cardiac injury. *Free Radical Biology and Medicine* 42(12): p.1818–1825. Available at:
 529 <https://pubmed.ncbi.nlm.nih.gov/17512461/> [Accessed December 30, 2020].
- 530 Dave, D.T., & Patel, B.M. 2019. Mitochondrial Metabolism in Cancer Cachexia: Novel Drug
 531 Target. *Current Drug Metabolism* 20(14): p.1141–1153. Available at:
 532 <https://pubmed.ncbi.nlm.nih.gov/31418657/> [Accessed December 30, 2020].
- 533 Eom, G.H., Nam, Y.S., Oh, J.G., Choe, N., Min, H.K., Yoo, E.K., et al. 2014. Regulation of
 534 acetylation of histone deacetylase 2 by p300/CBP-associated factor/histone deacetylase
 535 5 in the development of cardiac hypertrophy. *Circulation Research* 114(7): p.1133–
 536 1143. Available at: <https://pubmed.ncbi.nlm.nih.gov/24526703/> [Accessed May 7,
 537 2021].
- 538 Eom, G.H., & Kook, H. 2015. Role of histone deacetylase 2 and its posttranslational
 539 modifications in cardiac hypertrophy. *BMB Reports* 48(3): p.131–138. Available at:
 540 <https://pubmed.ncbi.nlm.nih.gov/25388210/> [Accessed December 30, 2020].
- 541 Fearon, K., Strasser, F., Anker, S.D., Bosaeus, I., Bruera, E., Fainsinger, R.L., et al. 2011.
 542 Definition and classification of cancer cachexia : an international consensus. *Lancet*
 543 *Oncology* 12(5): p.489–495. Available at: [http://dx.doi.org/10.1016/S1470-](http://dx.doi.org/10.1016/S1470-2045(10)70218-7)
 544 [2045\(10\)70218-7](http://dx.doi.org/10.1016/S1470-2045(10)70218-7).
- 545 Fedorova, O. V., Talan, M.I., Agalakova, N.I., Droy-Lefaix, M.T., Lakatta, E.G., & Bagrov,

- 546 A.Y. 2003. Myocardial PKC β 2 and the sensitivity of Na/K-ATPase to marinobufagenin
547 are reduced by cicletanine in Dahl hypertension. *Hypertension* 41(3 I): p.505–511.
548 Available at: <https://pubmed.ncbi.nlm.nih.gov/12623951/> [Accessed December 30,
549 2020].
- 550 Goyal, B.R., & Mehta, A.A. 2012. Benefi cial role of spironolactone, telmisartan and their
551 combination on isoproterenol-induced cardiac hypertrophy. *Acta Cardiologica* 67(2):
552 p.203–211. Available at: <https://pubmed.ncbi.nlm.nih.gov/22641978/> [Accessed
553 December 30, 2020].
- 554 Goyal, B.R., Mesariya, P., Goyal, R.K., & Mehta, A.A. 2008. Effect of telmisartan on
555 cardiovascular complications associated with streptozotocin diabetic rats. *Molecular and*
556 *Cellular Biochemistry* 314(1–2): p.123–131. Available at:
557 <https://pubmed.ncbi.nlm.nih.gov/18425420/> [Accessed December 30, 2020].
- 558 Goyal, B.R., Parmar, K., Goyal, R.K., & Mehta, A.A. 2011. Beneficial role of telmisartan on
559 cardiovascular complications associated with STZ-induced type 2 diabetes in rats.
560 *Pharmacological Reports* 63(4): p.956–966.
- 561 Goyal, B.R., Solanki, N., Goyal, R.K., & Mehta, A.A. 2009. Investigation into the cardiac
562 effects of spironolactone in the experimental model of type 1 diabetes. *Journal of*
563 *Cardiovascular Pharmacology* 54(6): p.502–509.
- 564 Hahn, C.-G., Umapathy, Wang, H.-Y., Koneru, R., Levinson, D.F., & Friedman, E. 2005.
565 Lithium and valproic acid treatments reduce PKC activation and receptor-G protein
566 coupling in platelets of bipolar manic patients. *Journal of Psychiatric Research* 39(4):
567 p.355–363. Available at:
568 <https://linkinghub.elsevier.com/retrieve/pii/S0022395604001396> [Accessed December
569 30, 2020].
- 570 Horton, J.W., Tan, J., White, D.J., & Maass, D.L. 2007. Burn injury decreases myocardial
571 Na-K-ATPase activity: Role of PKC inhibition. *American Journal of Physiology -*

- 572 *Regulatory Integrative and Comparative Physiology* 293(4). Available at:
573 <https://pubmed.ncbi.nlm.nih.gov/17634196/> [Accessed December 30, 2020].
- 574 Ji, M., Lee, E.J., Kim, K.B., Kim, Y., Sung, R., Lee, S.J., et al. 2015. HDAC inhibitors
575 induce epithelial-mesenchymal transition in colon carcinoma cells. *Oncology Reports*
576 33(5): p.2299–2308. Available at: [http://www.spandidos-](http://www.spandidos-publications.com/10.3892/or.2015.3879/abstract)
577 [publications.com/10.3892/or.2015.3879/abstract](http://www.spandidos-publications.com/10.3892/or.2015.3879/abstract) [Accessed December 30, 2020].
- 578 Kee, H.J., Sohn, I.S., Nam, K. Il, Park, J.E., Qian, Y.R., Yin, Z., et al. 2006. Inhibition of
579 histone deacetylation blocks cardiac hypertrophy induced by angiotensin II infusion and
580 aortic banding. *Circulation* 113(1): p.51–59. Available at:
581 <https://pubmed.ncbi.nlm.nih.gov/16380549/> [Accessed December 30, 2020].
- 582 Kee, H.J., Eom, G.H., Joung, H., Shin, S., Kim, J.R., Cho, Y.K., et al. 2008. Activation of
583 histone deacetylase 2 by inducible heat shock protein 70 in cardiac hypertrophy.
584 *Circulation Research* 103(11): p.1259–1269. Available at:
585 <https://pubmed.ncbi.nlm.nih.gov/18849323/> [Accessed May 7, 2021].
- 586 Knipstein, J., & Gore, L. 2011. Entinostat for treatment of solid tumors and hematologic
587 malignancies. *Expert Opinion on Investigational Drugs* 20(10): p.1455–1467. Available
588 at: <http://www.tandfonline.com/doi/full/10.1517/13543784.2011.613822> [Accessed
589 October 27, 2020].
- 590 Kong, Y., Tannous, P., Lu, G., Berenji, K., Rothermel, B.A., Olson, E.N., et al. 2006.
591 Suppression of class I and II histone deacetylases blunts pressure-overload cardiac
592 hypertrophy. *Circulation* 113(22): p.2579–2588. Available at:
593 <https://pubmed.ncbi.nlm.nih.gov/16735673/> [Accessed December 30, 2020].
- 594 Kook, H., & Kee, H.J. 2011. Roles and targets of class I and IIa histone deacetylases in
595 cardiac hypertrophy. *Journal of Biomedicine and Biotechnology* 2011.
- 596 Leus, N.G.J., Bosch, T. Van Den, Wouden, P.E. Van Der, Krist, K., Ourailidou, M.E.,
597 Eleftheriadis, N., et al. 2017. HDAC1-3 inhibitor MS-275 enhances IL10 expression in

- 598 reduces cigarette smoke-induced airway inflammation in mice. *Nature Publishing*
599 *Group* (February): p.1–18. Available at: <http://dx.doi.org/10.1038/srep45047>.
- 600 Li, J., Yao, Q., & Liu, D. 2011. Hydrodynamic cell delivery for simultaneous establishment
601 of tumor growth in mouse lung, liver and kidney. *Cancer Biology and Therapy* 12(8):
602 p.737–741.
- 603 Li, Y., & Seto, E. 2016. HDACs and HDAC inhibitors in cancer development and therapy.
604 *Cold Spring Harbor Perspectives in Medicine* 6(10). Available at:
605 [/pmc/articles/PMC5046688/?report=abstract](https://pubmed.ncbi.nlm.nih.gov/26579519/) [Accessed December 30, 2020].
- 606 Li, Z., & Langhans, S.A. 2015. Transcriptional regulators of Na,K-ATPase subunits.
607 *Frontiers in Cell and Developmental Biology* 3(OCT). Available at:
608 <https://pubmed.ncbi.nlm.nih.gov/26579519/> [Accessed December 30, 2020].
- 609 Lim, S., Brown, J.L., Washington, T.A., & Greene, N.P. 2020. Development and progression
610 of cancer cachexia: Perspectives from bench to bedside. *Sports Medicine and Health*
611 *Science*. Available at: <https://linkinghub.elsevier.com/retrieve/pii/S2666337620300664>
612 [Accessed December 26, 2020].
- 613 Liu, Z., Li, T., Liu, Y., Jia, Z., Li, Y., Zhang, C., et al. 2009. WNT signaling promotes
614 Nkx2.5 expression and early cardiomyogenesis via downregulation of Hdac1.
615 *Biochimica et Biophysica Acta - Molecular Cell Research* 1793(2): p.300–311.
616 Available at: <https://pubmed.ncbi.nlm.nih.gov/18851995/> [Accessed May 7, 2021].
- 617 López-Otín, C., & Matrisian, L.M. 2007. Emerging roles of proteases in tumour suppression.
618 *Nature Reviews Cancer* 7(10): p.800–808. Available at:
619 <https://pubmed.ncbi.nlm.nih.gov/17851543/> [Accessed December 30, 2020].
- 620 Matsumoto, T., Fujimoto-Ouchi, K., Tamura, S., Tanaka, Y., & Ishitsuka, H. 1999. Tumour
621 inoculation site dependent induction of cachexia in mice bearing colon 26 carcinoma.
622 *British Journal of Cancer* 79(5–6): p.764–769.
- 623 Montgomery, R.L., Davis, C.A., Potthoff, M.J., Haberland, M., Fielitz, J., Qi, X., et al. 2007.

- 624 Histone deacetylases 1 and 2 redundantly regulate cardiac morphogenesis, growth, and
 625 contractility. *Genes and Development* 21(14): p.1790–1802. Available at:
 626 <https://pubmed.ncbi.nlm.nih.gov/17639084/> [Accessed December 30, 2020].
- 627 Nojiri, H., Shimizu, T., Funakoshi, M., Yamaguchi, O., Zhou, H., Kawakami, S., et al. 2006.
 628 Oxidative stress causes heart failure with impaired mitochondrial respiration. *Journal of*
 629 *Biological Chemistry* 281(44): p.33789–33801. Available at:
 630 <https://pubmed.ncbi.nlm.nih.gov/16959785/> [Accessed December 30, 2020].
- 631 Nural-Guvener, H.F., Zakharova, L., Nimlos, J., Popovic, S., Mastroeni, D., & Gaballa, M.A.
 632 2014. HDAC class I inhibitor, Mocetinostat, reverses cardiac fibrosis in heart failure and
 633 diminishes CD90+ cardiac myofibroblast activation. *Fibrogenesis and Tissue Repair*
 634 7(1). Available at: <https://pubmed.ncbi.nlm.nih.gov/25024745/> [Accessed December 30,
 635 2020].
- 636 Olzscha, H., Sheikh, S., & La Thangue, N.B. 2015. Deacetylation of chromatin and gene
 637 expression regulation: A new target for epigenetic therapy. *Critical Reviews in*
 638 *Oncogenesis* 20(1–2): p.1–17.
- 639 Patel, B. 2019. *Histone deacetylases and oxidative stress: Role in diabetic cardiomyopathy.*
 640 *In: Oxidative Stress in Heart Diseases; eds. Prof. Sajal Chakraborti, Prof. Naranjan S*
 641 *Dhalla, Dr. Madhu Dixit, Prof. Nirmal K Ganguly.*
- 642 Patel, B.M. 2018. Sodium Butyrate Controls Cardiac Hypertrophy in Experimental Models of
 643 Rats. *Cardiovascular Toxicology* 18(1). Available at:
 644 <https://pubmed.ncbi.nlm.nih.gov/28389765/> [Accessed October 27, 2020].
- 645 Patel, B.M., Agarwal, S.S., & Bhadada, S. V. 2012. Perindopril protects against
 646 streptozotocin-induced hyperglycemic myocardial damage/alterations. *Human and*
 647 *Experimental Toxicology* 31(11): p.1132–1143.
- 648 Patel, B.M., & Bhadada, S. V. 2014. Type 2 diabetes-induced cardiovascular complications:
 649 Comparative evaluation of spironolactone, atenolol, metoprolol, ramipril and

- perindopril. *Clinical and Experimental Hypertension* 36(5): p.340–347. Available at: <https://pubmed.ncbi.nlm.nih.gov/24047125/> [Accessed December 30, 2020].
- Patel, B.M., Damle, D. 2013. Combination of telmisartan with cisplatin controls oral cancer cachexia in rats. *BioMed Research International* 2013: 10 pages Article ID 642848
- Patel, B.M., & Desai, V.J. 2014. Beneficial role of tamoxifen in experimentally induced cardiac hypertrophy. *Pharmacological Reports* 66(2): p.264–272.
- Patel, B.M., Kakadiya, J., Goyal, R.K., & Mehta, A.A. 2013. Effect of spironolactone on cardiovascular complications associated with type-2 diabetes in rats. *Experimental and Clinical Endocrinology and Diabetes* 121(8): p.441–447.
- Patel, B.M., Raghunathan, S., & Porwal, U. 2014. Cardioprotective effects of magnesium valproate in type 2 diabetes mellitus. *European Journal of Pharmacology* 728(1): p.128–134. Available at: <https://pubmed.ncbi.nlm.nih.gov/24530414/> [Accessed December 30, 2020].
- Patel, H.J., & Patel, B.M. 2017. TNF- α and cancer cachexia: Molecular insights and clinical implications. *Life Sciences* 170: p.56–63. Available at: <http://dx.doi.org/10.1016/j.lfs.2016.11.033>.
- Rabadiya, S., Bhadada, S., Dudhrejiya, A., Vaishnav, D., & Patel, B. 2018. Magnesium valproate ameliorates type 1 diabetes and cardiomyopathy in diabetic rats through estrogen receptors. *Biomedicine and Pharmacotherapy* 97: p.919–927.
- Raghunathan, S., Goyal, R.K., & Patel, B.M. 2016. Selective inhibition of HDAC2 by magnesium valproate attenuates cardiac hypertrophy. *Canadian Journal of Physiology and Pharmacology* 95(3): p.260–267.
- Raghunathan, S., Tank, P., Bhadada, S., & Patel, B. 2014. Evaluation of buspirone on streptozotocin induced type 1 diabetes and its associated complications. *BioMed Research International* 2014. Available at: <https://pubmed.ncbi.nlm.nih.gov/24563867/> [Accessed December 30, 2020].

- 676 Rayabarapu, N., & Patel, B.M. 2014. Beneficial role of tamoxifen in isoproterenol-induced
677 myocardial infarction. *Canadian Journal of Physiology and Pharmacology* 92(10):
678 p.849–857. Available at: <https://pubmed.ncbi.nlm.nih.gov/25243890/> [Accessed
679 December 30, 2020].
- 680 Reddy, B.S. 1999. Role of dietary fiber in colon cancer: An overview. In *American Journal*
681 *of Medicine*, Elsevier Inc. Available at: <https://pubmed.ncbi.nlm.nih.gov/10089109/>
682 [Accessed December 30, 2020].
- 683 Rishikof, D.C., Ricupero, D.A., Liu, H., & Goldstein, R.H. 2004. Phenylbutyrate decreases
684 type I collagen production in human lung fibroblasts. *Journal of Cellular Biochemistry*
685 91(4): p.740–748. Available at: <http://doi.wiley.com/10.1002/jcb.10742> [Accessed
686 December 30, 2020].
- 687 De Ruijter, A.J.M., Van Gennip, A.H., Caron, H.N., Kemp, S., & Van Kuilenburg, A.B.P.
688 2003. Histone deacetylases (HDACs): Characterization of the classical HDAC family.
689 *Biochemical Journal* 370(3): p.737–749. Available at:
690 <https://pubmed.ncbi.nlm.nih.gov/12429021/> [Accessed October 27, 2020].
- 691 Ryan, Q.C., Headlee, D., Acharya, M., Sparreboom, A., Trepel, J.B., Ye, J., et al. 2005.
692 Phase I and pharmacokinetic study of MS-275, a histone deacetylase inhibitor, in
693 patients with advanced and refractory solid tumors or lymphoma. *Journal of Clinical*
694 *Oncology* 23(17): p.3912–3922. Available at:
695 <https://pubmed.ncbi.nlm.nih.gov/15851766/> [Accessed October 27, 2020].
- 696 Sharma, B., Chaube, U., & Patel, B.M. 2019. Beneficial Effect of Silymarin in Pressure
697 Overload Induced Experimental Cardiac Hypertrophy. *Cardiovascular Toxicology*
698 19(1): p.23–35.
- 699 Souza, C., & Chatterji, B. 2015. HDAC Inhibitors as Novel Anti-Cancer Therapeutics.
700 *Recent Patents on Anti-Cancer Drug Discovery* 10(2): p.145–162. Available at:
701 <https://pubmed.ncbi.nlm.nih.gov/25782916/> [Accessed October 27, 2020].

- 702 Stevens, S.C.W., Velten, M., Youtz, D.J., Clark, Y., Jing, R., Reiser, P.J., et al. 2015.
703 Losartan treatment attenuates tumor-induced myocardial dysfunction. *Journal of*
704 *Molecular and Cellular Cardiology* 85: p.37–47. Available at:
705 <https://europepmc.org/articles/PMC4530048> [Accessed December 30, 2020].
- 706 Sukumaran, S., Patel, H.J., Patel, B.M. 2016. Evaluation of role of telmisartan in combination
707 with 5-fluorouracil in gastric cancer cachexia. *Life Sci.* 154: 15-23
- 708 Sun, R., Zhang, S., Hu, W., Lu, X., Lou, N., Yang, Z., et al. 2016. Valproic acid attenuates
709 skeletal muscle wasting by inhibiting C/EBP β -regulated atrogen1 expression in cancer
710 cachexia. *American Journal of Physiology - Cell Physiology* 311(1): p.C101–C115.
711 Available at: <http://ajpcell.physiology.org/lookup/doi/10.1152/ajpcell.00344.2015>.
- 712 Tian, M., Asp, M.L., Nishijima, Y., & Belury, M.A. 2011. Evidence for cardiac atrophic
713 remodeling in cancer-induced cachexia in mice. *International Journal of Oncology*
714 39(5): p.1321–1326. Available at: <https://pubmed.ncbi.nlm.nih.gov/21822537/>
715 [Accessed December 30, 2020].
- 716 Tsai, T.H., Chai, H.T., Sun, C.K., Yen, C.H., Leu, S., Chen, Y.L., et al. 2012. Obesity
717 suppresses circulating level and function of endothelial progenitor cells and heart
718 function. *Journal of Translational Medicine* 10(1). Available at:
719 <https://pubmed.ncbi.nlm.nih.gov/22747715/> [Accessed December 30, 2020].
- 720 Usui, T., Morita, T., Okada, M., & Yamawaki, H. 2014. Histone deacetylase 4 controls
721 neointimal hyperplasia via stimulating proliferation and migration of vascular smooth
722 muscle cells. *Hypertension (Dallas, Tex. : 1979)* 63(2): p.397–403. Available at:
723 <http://www.ncbi.nlm.nih.gov/pubmed/24166750> [Accessed October 27, 2020].
- 724 Wang, Y., Gao, B., & Xiong, S. 2014. Involvement of NLRP3 inflammasome in CVB3-
725 induced viral myocarditis. *American Journal of Physiology-Heart and Circulatory*
726 *Physiology* 307(10): p.H1438–H1447. Available at:
727 <https://www.physiology.org/doi/10.1152/ajpheart.00441.2014> [Accessed December 30,

- 2020].
- Wu, Y., Leng, Y., Meng, Q., Xue, R., Zhao, B., Zhan, L., et al. 2017. Suppression of Excessive Histone Deacetylases Activity in Diabetic Hearts Attenuates Myocardial Ischemia/Reperfusion Injury via Mitochondria Apoptosis Pathway. *Journal of Diabetes Research* 2017.
- Wysong, A., Couch, M., Shadfar, S., Li, L., Rodriguez, J.E., Asher, S., et al. 2011. NF- κ B inhibition protects against tumor-induced cardiac atrophy in vivo. *American Journal of Pathology* 178(3): p.1059–1068. Available at: [/pmc/articles/PMC3070568/?report=abstract](https://pubmed.ncbi.nlm.nih.gov/24898083/) [Accessed December 30, 2020].
- Zhang, J., & Zhong, Q. 2014. Histone deacetylase inhibitors and cell death. *Cellular and Molecular Life Sciences* 71(20): p.3885–3901. Available at: <https://pubmed.ncbi.nlm.nih.gov/24898083/> [Accessed December 30, 2020].
- Zhao, L., Chen, C.N., Hajji, N., Oliver, E., Cotroneo, E., Wharton, J., et al. 2012. Histone deacetylation inhibition in pulmonary hypertension: Therapeutic potential of valproic acid and suberoylanilide hydroxamic acid. *Circulation* 126(4): p.455–467. Available at: <https://pubmed.ncbi.nlm.nih.gov/22711276/> [Accessed December 30, 2020].
- Zhou, F.Q. 2005. Pyruvate in the Correction of Intracellular Acidosis: A Metabolic Basis as a Novel Superior Buffer. *American Journal of Nephrology* 25(1): p.55–63. Available at: <https://www.karger.com/Article/FullText/84141> [Accessed December 30, 2020].

Figure legends:

Figure 1: (A) Effect of HDAC inhibitors on hypertrophic markers in B16F1 induced cancer cachexia (i) cardiac hypertrophy index (ii) right ventricle weight (iii) left ventricle weight (iv) heart weight. Data represented in terms of (mean $\pm$ SEM, n=6). * significantly different than normal control (p<0.05), # significantly different than disease control (p<0.05). **(B)** Effect of HDAC inhibitors on hypertrophic markers in LLC induced cancer cachexia (i) cardiac hypertrophy index (ii) right ventricle weight (iii) left ventricle weight (iv) heart weight. Data represented in terms of (mean $\pm$ SEM, n=6). * significantly different than normal control (p<0.05), # significantly different than disease control (p<0.05). NC, Normal control mice; DC, Cancer cachexia (disease control) animals; EN, Cancer cachexia animals treated with Entinostat; MC, Cancer cachexia animals treated with MC1568; SB, Cancer cachexia animals treated with Sodium butyrate.

Figure 2: (A) Effect of HDAC inhibitors on cardiac markers in B16F1 induced cancer cachexia (i) collagen content in heart tissue (ii) serum creatinine kinase (CK-MB) (iii) serum lactate dehydrogenase (LDH) (iv) Na⁺K⁺ATPase activity in heart tissue (v) serum Troponin I . Data represented in terms of (mean $\pm$ SEM, n=6). * significantly different than normal control (p<0.05), # significantly different than disease control (p<0.05). **(B)** Effect of HDAC inhibitors on cardiac markers in LLC induced cancer cachexia (i) collagen content in heart tissue (ii) serum creatinine kinase (CK-MB) (iii) serum lactate dehydrogenase (LDH) (iv) Na⁺K⁺ATPase activity in heart tissue (v) serum Troponin I . Data represented in terms of (mean $\pm$ SEM, n=6). * significantly different than normal control (p<0.05), # significantly different than disease control (p<0.05). NC, Normal control mice; DC, Cancer cachexia (disease control) animals; EN, Cancer cachexia animals treated with Entinostat; MC, Cancer cachexia animals treated with MC1568; SB, Cancer cachexia animals treated with Sodium butyrate.

Figure 3: (A) Effect of HDAC inhibitors on hemodynamic markers in B16F1 induced cancer cachexia (i) mean blood pressure (ii) systolic pressure (iii) diastolic pressure (iv) heart rate (v)

774 rate of pressure development (vi) rate of pressure decay. Data represented in terms of (mean $\pm$
 775 SEM, n=6). * significantly different than normal control ($p<0.05$), # significantly different than
 776 disease control ($p<0.05$). **(B)** Effect of HDAC inhibitors on hemodynamic markers in LLC
 777 induced cancer cachexia (i) mean blood pressure (ii) systolic pressure (iii) diastolic pressure
 778 (iv) heart rate (v) rate of pressure development (vi) rate of pressure decay. Data represented in
 779 terms of (mean $\pm$ SEM, n=6). * significantly different than normal control ($p<0.05$), #
 780 significantly different than disease control ($p<0.05$). NC, Normal control mice; DC, Cancer
 781 cachexia (disease control) animals; EN, Cancer cachexia animals treated with Entinostat; MC,
 782 Cancer cachexia animals treated with MC1568; SB, Cancer cachexia animals treated with
 783 Sodium butyrate.

784 **Figure 4:** Representative images of heart tissue in B16F1 induced cancer cachexia at 400x
 785 resolution with **(A)** Periodic acid schiff (PAS) stain showing collagen in heart tissue in magenta
 786 colour **(B)** Masson trichrome (MT) staining differentiating cardiac muscle in red colour,
 787 collagen in blue colour and black coloured cell nuclei **(C)** Picro sirius red (SE) stain showing
 788 collagen in red colour with yellow coloured cardiac muscle fibres **(D)** Haematoxylin and Eosin
 789 (H&E) stain showing cardiac cellular architecture in blur-purple colour, extracellular matrix
 790 and cytoplasm in pink **(E)** Immunohistochemistry staining with vimentin in heart tissue. NC,
 791 Normal control mice; DC, Cancer cachexia (disease control) animals; EN, Cancer cachexia
 792 animals treated with Entinostat; MC, Cancer cachexia animals treated with MC1568; SB,
 793 Cancer cachexia animals treated with Sodium butyrate.

794 **Figure 5:** Representative images of heart tissue in LLC induced cancer cachexia at 400x
 795 resolution with **(A)** Periodic acid schiff (PAS) stain showing collagen in heart tissue in magenta
 796 colour **(B)** Masson trichrome (MT) staining differentiating cardiac muscle in red colour,
 797 collagen in blue colour and black coloured cell nuclei **(C)** Picro sirius red (SE) stain showing
 798 collagen in red colour with yellow coloured cardiac muscle fibres **(D)** Haematoxylin and Eosin
 799 (H&E) stain showing cardiac cellular architecture in blur-purple colour, extracellular matrix

800 and cytoplasm in pink (**E**) protein expression of caspase-3 in heart tissue in LLC induced cancer
801 cachexia as determined by western blotting, densitometric analysis of protein expressed in
802 arbitrary units normalized to GAPDH determined by using Image J. NC, Normal control mice;
803 DC, Cancer cachexia (disease control) animals; EN, Cancer cachexia animals treated with
804 Entinostat; MC, Cancer cachexia animals treated with MC1568; SB, Cancer cachexia animals
805 treated with Sodium butyrate.

Draft

Figure 1: (A) Effect of HDAC inhibitors on hypertrophic markers in B16F1 induced cancer cachexia (i) cardiac hypertrophy index (ii) right ventricle weight (iii) left ventricle weight (iv) heart weight. Data represented in terms of (mean $\pm$ SEM, n=6). * significantly different than normal control ($p < 0.05$), # significantly different than disease control ($p < 0.05$). (B) Effect of HDAC inhibitors on hypertrophic markers in LLC induced cancer cachexia (i) cardiac hypertrophy index (ii) right ventricle weight (iii) left ventricle weight (iv) heart weight. Data represented in terms of (mean $\pm$ SEM, n=6). * significantly different than normal control ($p < 0.05$), # significantly different than disease control ($p < 0.05$). NC, Normal control mice; DC, Cancer cachexia (disease control) animals; EN, Cancer cachexia animals treated with Entinostat; MC, Cancer cachexia animals treated with MC1568; SB, Cancer cachexia animals treated with Sodium butyrate.

406x406mm (300 x 300 DPI)

Figure 2: (A) Effect of HDAC inhibitors on cardiac markers in B16F1 induced cancer cachexia (i) collagen content in heart tissue (ii) serum creatinine kinase (CK-MB) (iii) serum lactate dehydrogenase (LDH) (iv) Na+K+ATPase activity in heart tissue (v) serum Troponin I . Data represented in terms of (mean $\pm$ SEM, n=6). * significantly different than normal control ($p < 0.05$), # significantly different than disease control ($p < 0.05$). (B) Effect of HDAC inhibitors on cardiac markers in LLC induced cancer cachexia (i) collagen content in heart tissue (ii) serum creatinine kinase (CK-MB) (iii) serum lactate dehydrogenase (LDH) (iv) Na+K+ATPase activity in heart tissue (v) serum Troponin I . Data represented in terms of (mean $\pm$ SEM, n=6). * significantly different than normal control ($p < 0.05$), # significantly different than disease control ($p < 0.05$). NC, Normal control mice; DC, Cancer cachexia (disease control) animals; EN, Cancer cachexia animals treated with Entinostat; MC, Cancer cachexia animals treated with MC1568; SB, Cancer cachexia animals treated with Sodium butyrate.

406x406mm (300 x 300 DPI)

Figure 3: (A) Effect of HDAC inhibitors on hemodynamic markers in B16F1 induced cancer cachexia (i) mean blood pressure (ii) systolic pressure (iii) diastolic pressure (iv) heart rate (v) rate of pressure development (vi) rate of pressure decay. Data represented in terms of (mean $\pm$ SEM, n=6). * significantly different than normal control (p<0.05), # significantly different than disease control (p<0.05). (B) Effect of HDAC inhibitors on hemodynamic markers in LLC induced cancer cachexia (i) mean blood pressure (ii) systolic pressure (iii) diastolic pressure (iv) heart rate (v) rate of pressure development (vi) rate of pressure decay. Data represented in terms of (mean $\pm$ SEM, n=6). * significantly different than normal control (p<0.05), # significantly different than disease control (p<0.05). NC, Normal control mice; DC, Cancer cachexia (disease control) animals; EN, Cancer cachexia animals treated with Entinostat; MC, Cancer cachexia animals treated with MC1568; SB, Cancer cachexia animals treated with Sodium butyrate.

406x406mm (300 x 300 DPI)

Figure 4: Representative images of heart tissue in B16F1 induced cancer cachexia at 400x resolution with (A) Periodic acid schiff (PAS) stain showing collagen in heart tissue in magenta colour (B) Masson trichrome (MT) staining differentiating cardiac muscle in red colour, collagen in blue colour and black coloured cell nuclei (C) Picro sirius red (SE) stain showing collagen in red colour with yellow coloured cardiac muscle fibres (D) Haematoxylin and Eosin (H&E) stain showing cardiac cellular architecture in blur-purple colour, extracellular matrix and cytoplasm in pink (E) Immunohistochemistry staining with vimentin in heart tissue. NC, Normal control mice; DC, Cancer cachexia (disease control) animals; EN, Cancer cachexia animals treated with Entinostat; MC, Cancer cachexia animals treated with MC1568; SB, Cancer cachexia animals treated with Sodium butyrate.

406x406mm (300 x 300 DPI)

Figure 5: Representative images of heart tissue in LLC induced cancer cachexia at 400x resolution with (A) Periodic acid schiff (PAS) stain showing collagen in heart tissue in magenta colour (B) Masson trichrome (MT) staining differentiating cardiac muscle in red colour, collagen in blue colour and black coloured cell nuclei (C) Picro sirius red (SE) stain showing collagen in red colour with yellow coloured cardiac muscle fibres (D) Haematoxylin and Eosin (H&E) stain showing cardiac cellular architecture in blur-purple colour, extracellular matrix and cytoplasm in pink (E) protein expression of caspase-3 in heart tissue in LLC induced cancer cachexia as determined by western blotting, densitometric analysis of protein expressed in arbitrary units normalized to GAPDH determined by using Image J. NC, Normal control mice; DC, Cancer cachexia (disease control) animals; EN, Cancer cachexia animals treated with Entinostat; MC, Cancer cachexia animals treated with MC1568; SB, Cancer cachexia animals treated with Sodium butyrate.

406x406mm (300 x 300 DPI)