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Research Article

Protective Effect of NQO-1 Modulator Quercetin in Hypertensive Rats

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ABSTRACT

Despite abundant anti-hypertensive therapies, there always remains an opportunity (perhaps need) to identify novel targets. One such emerging target is NAD(P)H:quinone oxidoreductase 1 (NQO-1), which acts downstream in Nrf2 cascade. This study was planned to investigate the effect of Nrf2 activator quercetin on NQO-1 modulation in hypertensive wistar albino rats. The hypertension was induced by DOCA (25 mg/kg s.c. twice weekly) and 1% NaCl in drinking water. The animals were randomized into six groups receiving either vehicle (control), DOCA- 1% NaCl salt (model) or treatments (Telmisartan (10 mg/kg) and quercetin (10, 25, 50 mg/kg) for 5 weeks. Various haemodynamic parameters, left ventricular functions, biological markers, lipid and protein peroxidative marker, antioxidant enzymes activity as well as histology (heart and kidney) were carried out. The expression of mRNA of NQO-1 gene in heart homogenate was estimated. Treatment with quercetin significantly prevented the rise in blood pressure, organ weight, improved left ventricular function, cardiac markers profile, kidney markers, restored antioxidant enzymes along with decrease in lipid and protein peroxidation. The normal architecture of heart and kidney were preserved in histopathological analysis by quercetin treatment. The upregulation of NQO-1 gene in heart by quercetin resulted in normal blood pressure. Altogether, quercetin prevented hypertension, improved cardiac function by augmenting the innate cell defense system in DOCA-salt rats. Such promising effects are attributed to novel cellular level target, NQO-1, which have multiple effects like regularisation of oxidative stress, eNOS activation and inhibition of ACE shedding.

Introduction

Cardiovascular diseases (CVDs) are generalized terminology used for various conditions affecting heart or blood vessels. According to world health organization (WHO), CVDs comprises of coronary heart disease, cerebrovascular disease, peripheral arterial disease, rheumatic heart disease, congenital heart disease, deep vein thrombosis and pulmonary embolism.[1] They accounts for at least three-quarters of the global deaths in low- and middle-income countries. The key to control CVDs lies in management of risk factors associated with them. One of the greatest risk factors which crosses its path in pathophysiology of above-mentioned CVDs is hypertension. Blood pressure is the force exerted by blood against the walls of blood vessels (arteries) when it is pumped by the heart. The higher the pressure, the harder the heart has to pump. According to WHO, 1.13 billion people globally are suffering from hypertension in which two-third of the cases are found in low- and middle-income countries.[2] The current study in India, grounded on a national-level blood pressure survey, reported 42.9% aged 18–19 years had prehypertension.[3] Although, many anti-hypertensive medicines are available but they come with the price of various side effects.[4] Also, in actual world, unfortunately high blood pressure is managed in only 34% of patients and none of the available drug target root causes of hypertension at genetic level. The world is transitioning towards plant-based medicines because of the safety, efficacy, cultural acceptability and lesser side effects.[5] According to "WHO global report on traditional

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and complementary medicine 2019", plant-based medicine can make a noteworthy contribution to achieve the goal of universal health coverage by being included in people-centered health system that balances curative services with preventive care.[6] The rationale for the use of plant-based therapy is not at all surprising, considering the fact that they have bounty of bioactive constituents of therapeutic interest. The plants may target different molecular aspects of multicausal pathophysiology of hypertension *viz*. inhibition of RAAS pathway, increased NO, decrease inflammatory markers, elevate dilatory prostaglandins, normalization of adrenal and endothelin pathway, reactive oxygen species (ROS) scavenging and increase of cellular antioxidants.[5] Our molecule of interest is well-known antihypertensive and antioxidant polyphenolic flavonoid, quercetin. The role of oxidative stress in diseases like hypertension is well documented. The phase II metabolic enzymes are a battery of critical proteins that detoxify xenobiotics and are emerging target of reducing oxidative stress. This study focuses on one of the major phase II detoxification systems, NAD(P)H:quinone oxidoreductase (NQO1). NQO1 is one of the most noteworthy enzymes in cellular protection owing to its ability to catalyse reactive quinones and quinone imines to their less reactive as well as toxic hydroquinones forms.[7] Several *in-vitro* studies have reported upregulation of nuclear factor erythroid 2-related factor (NRF2)-antioxidant response element (ARE) system by Quercetin and its analogues.[8, 9] Nrf2 is ubiquitously and constitutively expressed by cells, ensuring prompt protective action against oxidative, inflammatory, and metabolic stresses. With activation of NRF-2, the downstream cascade of antioxidant genes like heme oxygenase-1 (HO-1), NQO1, and glutamate-cysteine ligase modifier subunits are also stimulated.[8, 10] The *in-vitro* studies have proven NQO-1 activation by quercetin. Valerio *et al.* proved increased NQO1 transcription in response to quercetin in MCF-7 human breast cancer cell line.[11] The *in-vivo* study conducted in adult mice concluded that quercetin increased protein and gene expression of NQO-1 gene in liver.[12] With this background, the functional study was aimed to test the hypothesis that NRF2 activator quercetin may modulate downstream NQO-1 gene and normalises the elevated blood pressure in rats.

MATERIALS AND METHODS

Materials

Procurement of Drug

Quercetin was procured from Sigma Aldrich Pvt. Ltd. Telmisartan was procured from Torrent Research Centre, Ahmedabad.

Chemicals and Kits

Bovine albumin, DOCA-salt, epinephrine, thiobarbituric acid (TBA), trichloroacetic acid (TCA), tris buffer, 5-5'-dithiobis [2-nitrobenzoic acid] (DTNB, Ellman's Reagent), 5-thionitrobenzoic acid (TNB), TRIazol, used in the study were of analytical grade and procured from Sigma-aldrich Pvt. Ltd. Ethylenediaminetetraacetic acid (EDTA), carboxy methyl cellulose (CMC), sodium citrate, citric acid, potassium dihydrogen phosphate (KH_2PO_4), sodium bicarbonate ($NaHCO_3$), potassium chloride (KCl), and glucose were of analytical grade and were obtained from Merck Pvt. Ltd, Hyderabad. Calcium chloride ($CaCl_2$), magnesium sulphate ($MgSO_4$), sodium chloride (NaCl), and sodium pyruvate were of analytical grade and were obtained from Chemdyes Cop., Rajkot. All the biochemical assay was performed using standard kits (creatine kinase-muscle/Brain (CK-MB), lactate dehydrogenase (LDH), electrolytes (Na^+ and K^+ and Ca^{+2}), creatinine, blood urea nitrogen (BUN), and uric acid), which were procured from i-chem Jeev Diagnostic Pvt. Ltd, Chennai. Primers were purchased from Eurofins Genomics, Bangalore. qRT-PCR Kit was purchased from applied biosystems, Thermoscientific Inc., Ahmedabad.

Experimental Procedures

Selection of Dose

The dose of Telmisartan (10 mg/kg) and three doses of quercetin (10 mg/kg, 25 mg/kg and 50 mg/kg) were selected from literature.[13]

Induction of Hypertension

Twelve weeks old healthy male wistar rats weighing 150–180 g were housed in a group of 6 rats per cage under well-controlled conditions of temperature (22 ± 2°C), humidity (55 ± 5 %), and 12/12 hours light-dark cycle. The animals had free access to conventional laboratory diet (purchased from Pranav Argo Pvt. Ltd) and distilled water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) of Anand Pharmacy College, Anand as per the guidance of the committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India (Protocol no. APC/2018-IAEC/1912).

Hypertension was induced in rats by the deoxycorticosterone acetate (DOCA)-salt.[14] The animals were randomized based on baseline parameter in six groups with eight animals each as follows:

Group I- Normal control; administered distilled water.
Group II- DOCA Model; administered 1% NaCl + DOCA-salt (25 mg/kg s.c. twice weekly)
Group III- Standard control- Tel 10; administered 1% NaCl + DOCA-salt + Std. drug Telmisartan (10 mg/kg, p.o.)
Group IV-VI: Q 10, Q 25, Q 50, respectively; administered 1% NaCl + DOCA-salt + Quercetin (10 mg/kg, 25 mg/kg and 50 mg/kg p.o., respectively)

DOCA was prepared in olive oil and was administered s.c. in the dose of 25 mg/kg twice weekly, for 5 weeks. The rats

Table 1: Effect of quercetin on organ weights and left ventricular function

	Normal Control	*DOCA Model*	*Tel 10*	*Q 10*	*Q 25*	*Q 50*
Heart weight/BW ratio (mg/g)	3.371 ± 0.005	5.286 ± 0.004 (##)	3.286 ± 0.001 (**)	4.389 ± 0.002	3.866 ± 0.001 (*)	3.707 ± 0.001 (**)
Kidney weight/BW ratio (mg/g)	4.906 ± 0.004	7.998 ± 0.004 (###)	5.352 ± 0.003 (**)	6.716 ± 0.003	5.706 ± 0.001 (*)	5.610 ± 0.003 (**)
LVEDP (mm Hg)	1.210 ± 0.400	4.723 ± 0.210 (###)	1.014 ± 0.095 (**)	3.125 ± 0.279	2.872 ± 0.1811 (*)	1.017 ± 0.275 (**)
$LVdp/dt_{max}$ (mm Hg/s)	10.520 ± 0.591	4.781 ± 0.647 (#)	9.785 ± 0.546 (*)	5.481 ± 1.587	7.853 ± 1.482	9.184 ± 1.248 (*)
$LVdp/dt_{min}$ (mm Hg/s)	-17.530 ± 1.210	-2.782 ± 0.624 (###)	-12.175 ± 1.610 (**)	-6.243 ± 1.241	-8.254 ± 1.712	-11.850 ± 1.421 (**)
CFR (ml/min)	20.460 ± 0.610	12.524 ± 1.270 (###)	18.927 ± 0.748 (**)	14.521 ± 1.324	16.851 ± 1.620 (*)	18.627 ± 1.420 (**)

Abbreviations: BW, body weight; DOCA model, DOCA-salt hypertensive rats; Tel, telmisartan treated rats; Q, Quercetin treated rats; LVEDP, left ventricular end diastolic pressure; $LVdp/dt_{max}$ and $LVdp/dt_{min}$, maximal rate of pressure rise and fall; CFR, Coronary flow rate.
Values are expressed as mean ± sem.
#$p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ model control vs. normal control
*$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ treatment control vs. model control

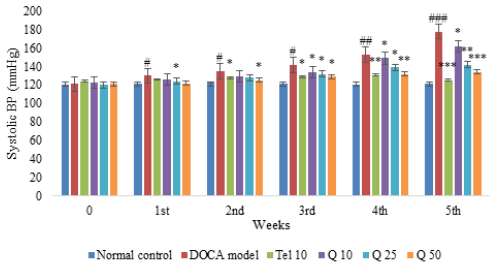

Fig. 1: Effect of Quercetin on systolic blood pressure
Values are expressed as mean±sem.
# $p < 0.05$, ## $p < 0.01$, and ### $p < 0.001$ model control vs. normal control
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ treatment control vs. model control

were given 1% w/v sodium chloride (NaCl) salt in drinking water. During the study duration, body weight of animals was recorded till the end of the experiment. The blood pressure (systolic, diastolic, mean arterial blood pressure) and electrocardiogram (ECG) were recorded every week using Biopac Student Lab (MP-36 Biopac Systems, Inc). At the end of study duration, blood samples were collected in clean dry centrifuge tubes from the retro-orbital plexuses under anaesthesia. Serum was separated and was analyzed for biochemical parameters like creatine kinase-muscle/Brain (CK-MB), lactate dehydrogenase (LDH), electrolytes (Na^+ and K^+ and Ca^{+2}), creatinine, blood urea nitrogen (BUN), and uric acid using a standard kit. At the end, the animals were humanely euthanized and organs (heart and kidney) were dissected out. The heart was mounted for Langendorff study[15] and $LVdp/dt_{max}$, $LVdp/dt_{min}$, left ventricular end diastolic pressure (LVEDP) as well as coronary flow reserve (CFR) were measured. A portion of heart was preserved for hematoxylin-eosin staining. The kidney was also stained with hematoxylin-eosin for assessment of various parameters like atrophy of tubular cell, necrosis, and glomerulus congestion and vacuolization The portion of heart was used for gene expression of NQO-1. MDA, GSH, SOD, Catalase and Advanced Oxidation of Protein Products (AOPP) levels were measured in heart homogenate.

Statistical analysis

Results were presented as mean ± SEM. Statistical analysis was done by using one-way ANOVA followed by Dunnett's post hoc test using Graph Pad prism v6.01. Data was considered statistically significant at $p<0.05$.

RESULT

Effect of Quercetin on Anthropometric Parameters

The body weight of animals remained non-significantly different from normal control throughout the duration suggesting DOCA and treatment group have no effect on normal growth of rats.
A significant ($p < 0.05$) increase in heart weight/100 g body weight ratio and right kidney weight/100 g body weight ratio was observed in DOCA control rats as compared to the control group suggesting hypertrophy of organs. The standard control and treatment groups significantly ($p < 0.05$) prevented increase in heart and kidney weight ratio with respect to body weight (Table 1).

Effect of Quercetin on Hemodynamic Parameters

The model rats showed significant increase in systolic blood pressure (SBP) and Diastolic blood pressure (DBP) over the study duration of five weeks when compared to normal group. At the end of 5 weeks, the blood pressure touched the height of 158/142 mmHg. (Fig. 1-2)
The standard group treated with telmisartan significantly ($p < 0.05$) prevented rise in blood pressure during the experimental period as compared to model control animals. The highest blood pressure reading in Tel 10 group was 132/96 mmHg. All treatment group showed significant ($p < 0.05$) inhibition of rise in blood pressure as compared to model control animals.

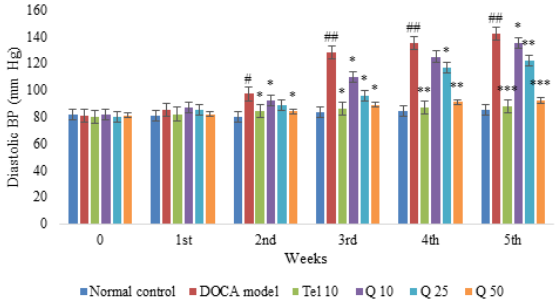

Fig. 2: Effect of Quercetin on diastolic blood pressure
Values are expressed as mean ± sem.
#$p < 0.05$, ##$p < 0.01$ model control vs. normal control
*$p < 0.05$, **$p < 0.01$, *** $p < 0.001$ treatment control vs. model control

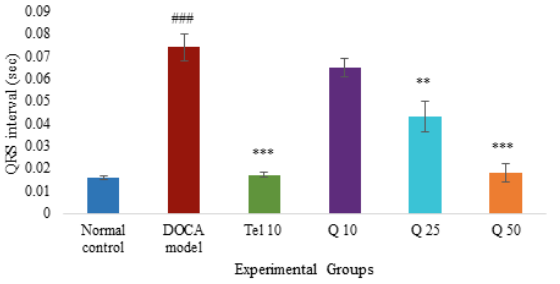

Fig. 4: (a) Effect of Quercetin on duration of QRS complex
Values are expressed as mean ± sem.
###$p < 0.001$ model control vs. normal control
**$p < 0.01$, *** $p < 0.001$ treatment control vs. model control

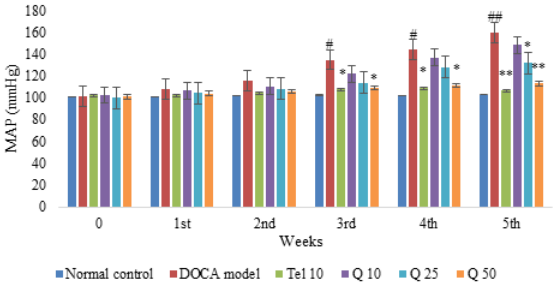

Fig. 3: Effect of Quercetin on mean arterial pressure
Values are expressed as mean ± sem.
#$p < 0.05$, ##$p < 0.01$ model control vs. normal control
*$p < 0.05$, ** $p < 0.01$ treatment control vs. model control

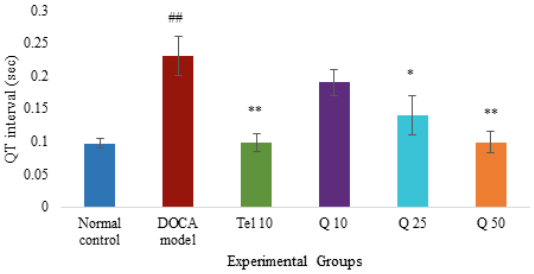

Fig. 4 (b) Effect of Quercetin on QT interval
Values are expressed as mean ± sem.
##$p < 0.01$ model control vs. normal control
*$p < 0.05$, ** $p < 0.01$ treatment control vs. model control

However, the best inhibition was seen with highest dose of Quercetin- Q 50. The blood pressure recordings of Q10, Q 25 and Q 50 at the end of the experiment were 148/135, 140/122, 134/92 mmHg, respectively. Also, the mean arterial pressure was gradually increased from week 1 to week 5 in DOCA-salt rats as compared to normal control rats, however quercetin treated rats attenuated increased mean arterial pressure in DOCA-salt treated rats at 5 weeks. (Fig. 3)

The ECG displayed differences in the normal, model, and treatment control groups (Fig. 4). A prolonged QRS (Fig. 4a) and QT interval (Fig. 4b) duration was noticed in the DOCA-salt treated rats ($p < 0.001$ and $p < 0.01$, respectively) proposing higher occurrence of arrhythmogenic events than normal control rats. This changes in interval duration were normalized significantly ($p < 0.001$ and $p < 0.01$, respectively) by telmisartan and higher dose of quercetin compared with model control rats.

Effect of Quercetin on Left Ventricular Function

The model group showed a significant increase in left ventricular end diastolic pressure (LVEDP) when compared to normal group. LVEDP was significantly decreased by Tel 10 ($p < 0.01$), mid ($p < 0.05$) and high ($p < 0.01$) doses of quercetin. Accordingly, $LVdp/dt_{max}$ ($p < 0.05$) and $LVdp/dt_{min}$ ($p < 0.001$) was significantly reduced in rats administrated with DOCA-salt compared with control. The highest dose of quercetin significantly reversed effect on these two parameters suggesting protective effect on heart by regularising cardiac function. CFR was significantly reduced in rats administrated with DOCA-salt compared with control while it was higher in Tel 10 ($p < 0.01$), mid ($p < 0.05$) and high ($p < 0.01$) doses of Quercetin compared with hypertensive group (Table 1).

Effect of Quercetin on Serum Biological Markers

The activities of tissue damage markers, CK-MB and LDH were significantly ($p < 0.05$) increased in DOCA salt administered hypertensive rats. The rats treated with standard and quercetin showed significant ($p < 0.05$) decrease in CK-MB and LDH. It is worth noting that standard and Q 50 treated animals showed near to normal levels of these enzymes (Table 2).

DOCA control rats showed a significant ($p < 0.05$) increase in serum sodium (Na^+) and calcium (Ca^{2+}) level and decrease in serum potassium (K^+) level as compared to control group. The Tel 10 and Quercetin treated animals showed significant decrease in Na^+ and Ca^{+2} ions. The levels of K^+ was significant increased in all treatment groups. The levels of Na^+ and Ca^{+2} was brought back to normal by standard and highest dose of quercetin (Table 2).

The kidney markers namely creatinine, BUN and uric acid were significantly increased in animals administered with DOCA and 1% w/v NaCl in drinking water as compared to

Table 2: Effect of quercetin on cardiac and renal markers

	Normal Control	*DOCA Model*	*Tel 10*	*Q 10*	*Q 25*	*Q 50*
CK-MB (U/L)	172 ± 36.930	602.940 ± 72.930 (###)	180.290 ± 039 (***)	479.420 ± 061 (*)	269.670 ± 059 (**)	190.890 ± 042 (***)
LDH (U/L)	239 ± 30.770	652.710 ± 100.600 (###)	249.200 ± 25.900 (***)	598.800 ± 87.090	324.100 ± 60.720 (**)	261.600 ± 40.500 (***)
Sodium (mM/L)	135 ± 11.200	179.700 ± 21.400 (##)	139.200 ± 13.800 (**)	168.900 ± 20.300	151.820 ± 17.000 (*)	144.500 ± 12.700 (**)
Potassium (mM/L)	6.200 ± 0.200	3.570 ± 0.390 (##)	5.950 ± 0.240 (**)	4.160 ± 0.340	4.790 ± 0.300 (*)	5.740 ± 0.280 (**)
Calcium (mg/dL)	10.100 ± 1.400	93.400 ± 6.000 (###)	12.300 ± 2.000 (***)	61.700 ± 5.000 (**)	27.900 ± 4.100 (**)	15.210 ± 3.000 (***)
Creatinine (mg/dL)	0.8 ± 0.007	2.790 ± 0.06 (##)	0.980 ± 0.013 (***)	2.670 ± 0.040 (*)	2.120 ± 0.070 (*)	1.140 ± 0.040 (***)
Uric acid (mg/dL)	1.37 ± 0.090	4.200 ± 0.833 (##)	1.450 ± 0.339 (***)	3.100 ± 0.658 (*)	2.500 ± 0.919 (*)	1.560 ± 0.6 00 (***)
BUN (mg/dL)	17.2 ± 2.659	42.410 ± 6.232 (##)	18 ± 1.244 (***)	31.500 ± 3.366 (*)	23.400 ± 2.584 (*)	19.300 ± 1.730 (***)

Abbreviations: CK-MB, Creatine kinase; LDH, Lactate dehydrogenase; BUN, Blood urea nitrogen.
Values are expressed as mean ± sem.
##p < 0.01 and ###p < 0.001 model control vs. normal control
*p < 0.05, **p < 0.01, ***p < 0.001 treatment control vs. model control

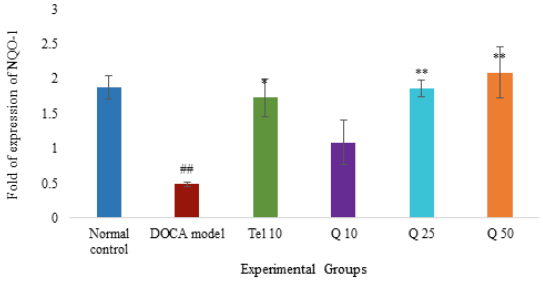

Fig. 5: Effect of Quercetin on NQO-1 gene expression
Values are expressed as mean ± sem.
##p < 0.01 model control vs. normal control
*p < 0.05, **p < 0.01 treatment control vs. model control

normal control animals. The kidney marker levels were significantly decreased in treatment groups as compared to model animals. The levels in Tel 10 and Q 50 treated animals were similar to that of normal control animals. (Table 2)

Effect of Quercetin on Lipid Peroxidation, AOPP and Endogenous Antioxidant Enzymes

The lipid peroxidation was measured in terms of MDA level. The levels of MDA were significantly (p < 0.001) higher in hypertensive rats as compared to normal control animals. The levels of advanced oxidation of protein products, a marker of protein oxidation, was also significantly (p < 0.05) increased in model control animals. Both of the oxidative stress markers were significantly decreased in Tel 10 and quercetin treated groups as compared to model control. (Table 3)

The antioxidant enzymes namely GSH (p<0.001), SOD (p < 0.01) and catalase (p < 0.05) were significantly decreased in model control animals as compared to normal group. The status of all these enzymes were preserved by treatment groups and significantly increased in treatment groups as compared to model control animals. (Table 3)

Effect of Quercetin on NQO-1 Gene Expression

The gene expression study revealed significant downregulation of NQO-1 gene in DOCA-salt fed hypertensive rats as compared to control group animals. The treatment with quercetin (Q10, Q 25 and Q 50) significantly averted DOCA effect by change in 1.21, 2.81, and 3.27 fold, respectively. Tel 10 also exhibited significant increase in NQO-1 gene expressions as compared to model control animals. (Fig. 5)

Effect of Quercetin on Heart and Kidney Histopathology

Histopathological examination of the heart tissues (Fig. 6a) showed normal tissue architecture with well-defined shape of all normal structures in normal control group animals. In DOCA-salt treated animals, severe myocardial degeneration, alteration in shape of myofibers, hypertrophy, and infiltration of inflammatory cells were observed as compared to normal group animals. Tel 10 showed the protective effects against myocardial degeneration, hypertrophy, and infiltration of inflammatory cells. Q 10 and Q 25 group of animals displayed less myocardial degeneration and altered shape of myofiber than the model control. High dose of quercetin showed a significant protection against DOCA-salt induced heart histopathological alterations as seen with telmisartan treated animals. The kidney histopathological examination (Fig. 6b) revealed necrosis, tubular damage (including atrophy and loss of brush border) along with an accumulation of interstitial matrix and evidence of

Table 3: Effect of quercetin on oxidative stress markers

	Normal Control	*DOCA Model*	*Tel 10*	*Q 10*	*Q 25*	*Q 50*
MDA (µg/mg protein)	0.016 ± 0.059	0.082 ± 0.068 (###)	0.017 ± 0.011 (***)	0.061 ± 0.049 (*)	0.035 ± 0.052 (**)	0.018 ± 0.006 (***)
SOD (unit/min/g tissue)	169 ± 21.780	56 ± 22.590 (##)	159 ± 13.470 (***)	97 ± 5.340 (*)	124 ± 14.380 (*)	147 ± 13.980 (***)
GSH (µg/mg protein)	1.970 ± 0.147	0.300 ± 0.003 (###)	1.620 ± 0.091 (***)	0.500 ± 0.003	1.340 ± 0.003 (**)	1.560 ± 0.028 (***)
Catalase (µM of H_2O_2 / min/g tissue)	92 ± 0.064	71 ± 0.261 (#)	89 ± 0.211 (*)	76 ± 0.217	80 ± 0.358	86 ± 0.184 (*)
AOPP (nM/mg protein)	0.210 ± 0.007	0.392 ± 0.060 (#)	0.252 ± 0.013 (**)	0.340 ± 0.040	0.280 ± 0.07 (*)	0.274 ± 0.004 (**)

Abbreviations: MDA, malondialdehyde; SOD, superoxide dismutase; GSH, glutathione; AOPP, advanced oxidation of protein products.
Values are expressed as mean ± sem.
#$p < 0.05$ and ###$p < 0.001$ model control vs. normal control
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ treatment control vs. model control

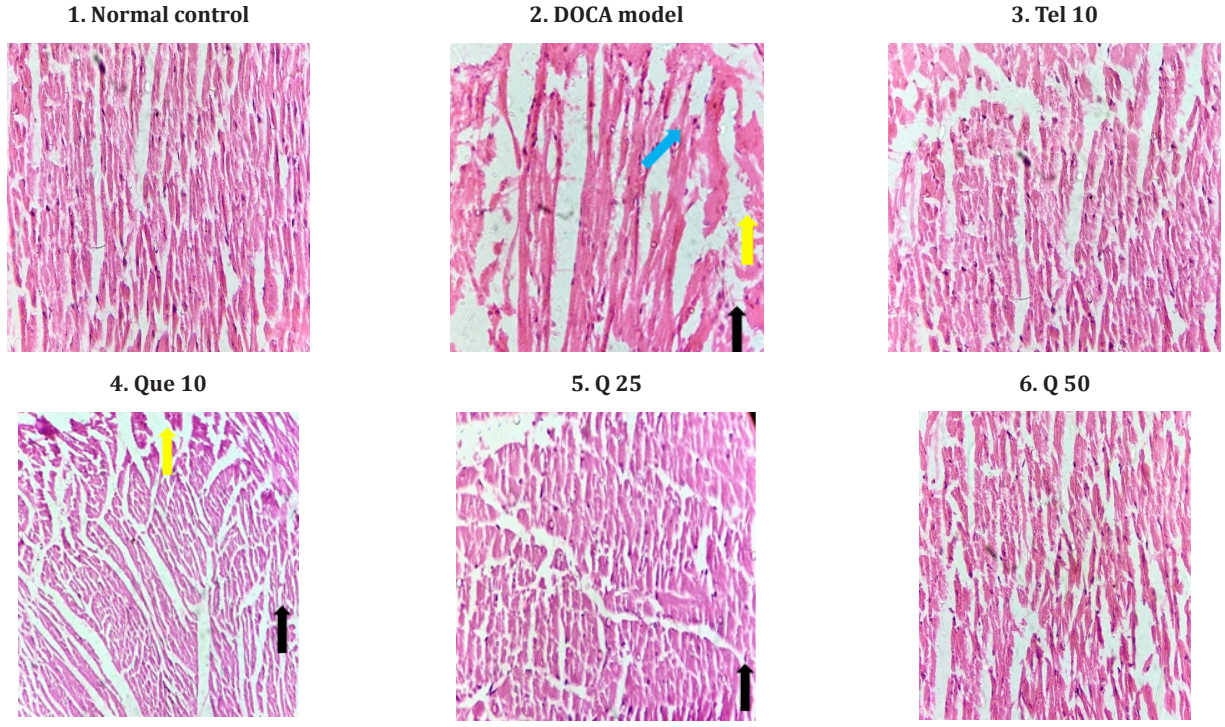

Fig. 6: (A) Effect of Quercetin on heart histopathology:
1) Normal control animals indicated normal tissue architecture with well-defined shape of myocardial cells and normal muscle fibres. 2) Model control animals showed myocardial degeneration (yellow arrow), altered structure of myofiber (blue arrow), and infiltration of inflammatory cells (black arrow).
3) Tel 10 receiving animals showed notable decrease in myocardial degeneration and preserve the normal architecture of myofiber.
4) Q 10 receiving animals indicated less myocardial degeneration (yellow arrow) and infiltration of inflammatory cells (black arrow).
5) Q 25 and Q 50 receiving animals displayed significant improvement in altered structure of heart as compared to model control group. (Magnification 40x)

inflammation in the cortex and glomerulus congestion were observed in DOCA-salt treated animals as compared to control. Tel 10 and Q 50 displayed maximum protection against the hypertensive damage by reducing atrophy, necrosis and tubular damage. Even though, Q 10 and Q 25 treated rats also demonstrated some protection but still modifications in kidney architecture were evident.

DISCUSSION

This present study examines the effect of quercetin in the animal model of hypertension induced by synthetic mineralocorticoid derivative DOCA-salt. Administration of DOCA creates an imbalance in renal sodium handling, where larger amounts of sodium and water are reabsorbed by the kidney. Additionally, the model integrates a high-salt

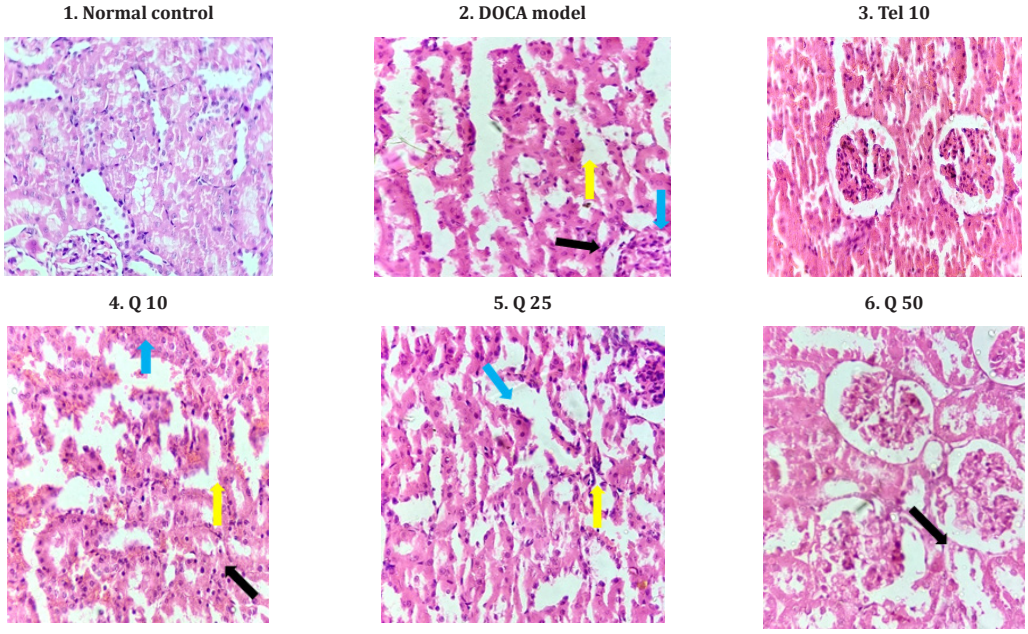

Fig. 6: (B) Effect of Quercetin on kidney histopathology:
1) Normal control animals indicated normal histology of kidney.
2) Model control animals showed glomerulus congestion (blue arrow), atrophy & necrosis (yellow arrow), tubular damage along with an accumulation of interstitial matrix and evidence of inflammation in the cortex (black arrow).
3) Tel 10 receiving animals showed less glomerulus congestion, necrosis, and tubular damage.
4) Q 10 receiving animals indicated slight improvement in glomerulus congestion (blue arrow), atrophy (yellow arrow), and tubular damage (black arrow).
5) Q 25 receiving animals showed tubular inflammation (blue arrow) and necrosis (yellow arrow). 6) Q 50 receiving animals indicated increase in number of glomerulus and at few places inflammation of renal tubules (black arrow) was evident. (Magnification 40x)

regime consisting of 1% NaCl in the drinking water. Salt sensitivity might be an important indicator in individuals who are predisposed to the development of hypertension. The amalgamation of DOCA-salt and increased salt intake results in chronic high BP developing in distinct stages. Also, DOCA-salt rats mimic most of the characteristics involved in cardiovascular remodelling in humans including hypertension, hypertrophy, fibrosis, conduction abnormalities and endothelial dysfunction. It also provides a suitable model to permit the assessment of various molecules possessing anti-oxidant property with respect to their effects on cardiovascular diseases.[16]

In the present study, hypertensive rats were successfully developed by DOCA-salt. The plethora of *in-vitro* and *in-vivo* studies have shown quercetin to be direct vasodilator. [17-19] The studies have also shown its metabolites to possess the same effect. This effect is also been reproduced in humans and that too in greater amount as compared to animal studies. [20] This vasodilatory effect might contribute to keep the blood pressure of rats in normal range. Our results are in line with the documented studies. All the three doses of quercetin successfully reduced elevated blood pressure.

The effect of quercetin on electrical activity of heart resembled the controls rather that DOCA-salt group. Prolonged QRS complex and QT interval in ECG leads is very common in DOCA-salt treated rats indicating cardiac hypertrophy which in turn induced the hypertension.[21] In this study, rats treated with DOCA-salt speeded the conduction of electrical impulses through the hearts and added the extra duration of the QRS complex, which increased the rate of contraction of the heart. It is viewed that salt overloading also be part of the cause to increased arrhythmogenesis events.[22] Mid and high dose quercetin treated rats correct the altered QRS complex and QT interval, which directs the protective activity against hypertension.

Numerous experiments have shown hypertrophy of heart during and post DOCA administration, indicated by alterations in cardiac functions. DOCA-salt treated animals are found to have thickened left ventricular wall as well as mass. Consequently, they manifested rising LVEDP as well as compromised systolic function (depicted by +dp/dt) and diastolic function displayed (depicted by -dp/dt).[23] In our experiment, all these effects were observed in model control animals. The effects were put to halt by highest dose of quercetin. This might be attributed to its antioxidant property which checked cardiac remodelling. Several studies documented that hypertension induced by DOCA-salt is always associated with significant hypertrophy of kidney and heart.[14, 24] Our study also noticed increase in heart and kidney weight in model control animals. Treatment with quercetin diminished increase in organ weight associated with hypertrophy and

thereby confirm prevention of hypertrophy. This might be due to its antihypertensive effect.

Myocardium contains a good number of diagnostic markers which are released once cells are damaged. The most widely used cardiac diagnostic markers are CK-MB and LDH, which are specific and sensitive enzymes.[25] Our present study also confirms the significant elevation of these diagnostic marker enzymes in serum in animals treated with DOCA. This might be due to increase in blood pressure which causes myocardial damage. Our molecule showed antihypertensive activity and thus how, it prevented damage to myocardial cells.

According to pathophysiology of hypertension, there is change in concentration of ions across the membrane. The sodium ions are increased while potassium ions are decreased intracellularly.[26] This consequently effects sympathetic activity along with various structural changes in vasculature.[27] The exact scenario was mimicked in our study. DOCA treated animals showed increased sodium ions along with calcium ions. The reduction of potassium ions intracellularly was also observed. Also, the increase in blood pressure has direct effect on kidney. The kidney markers namely creatinine, and BUN were increased in model control animals. Quercetin treated rats showed reversal of all these conditions owing to its antihypertensive activity.

As mentioned before, DOCA-salt induced hypertension is very well connected to oxidative stress and inflammation.[14,16] Clinically also, there is association of hypertension and oxidative stress. The present experiment showed increase in MDA level which is due to lipid peroxidation in DOCA treated animals. Also, the marker of protein oxidation, AOPP was found to be increased in model control. The antioxidant enzymes (GSH, SOD and Catalase) could not scavenge the oxidative products as the levels were declined in model control animals. The molecule of interest in present study, quercetin is well known antioxidant. And thus, in present study oxidative products were observed in smaller amount and antioxidant enzyme status was preserved. When the pathway for its antioxidant activity was deduced, it was observed to be activation of NRF2-ARE system. The present study aimed to find downstream gene(s) in the cascade. It was deduced to be upregulation of NAD(P)H:quinone oxidoreductase (NQO-1) gene. NQO-1 is the phase II enzyme that catalyses the oxidation of NAD(P)H to NAD(P)+ by various quinines. Interestingly, study conducted by Yong-Hoon Kim *et al.* showed activation of NQO-1 regulates ACE shedding in hypertensive rats.[28] ACE is a type-I membrane protein which undergoes ectodomain shedding to produce an enzymatically active soluble form.[28] Also, improvement of endothelial dysfunction due to eNOS activation was observed after NQO-1 gene upregulation.[29] In present study, quercetin upregulated NQO-1 gene expression and thus by mitigate ACE shedding and modulate blood pressure. Thus, it also inhibits ACE catalysed conversion of angiotensin I to angiotensin II.

In the heart histopathological studies of DOCA-salt control group animals presented myocardial degeneration, hypertrophy, and infiltration of inflammatory cells. These changes may attribute to the action of endothelin which evoked by endothelin type A receptor, a key mediator of hypertrophy. It is proposed that due to inflammation and oxidative stress more collagen deposition is occurring which may end up with cardiac fibrosis.[14, 30] The renal histopathology revealed necrosis and tubular damage (including atrophy and loss of brush border) along with an accumulation of interstitial matrix and evidence of inflammation in the cortex and glomerulus congestion in the model group which indicates kidney atrophy. Excess salt intake might also impair renal function to induce hypertrophy.[31] Concurrent treatment with quercetin, suppression of oxidative stress showed protective effect on these substantial changes proving its antihypertensive effect.

In conclusion, the unveiling of the current study confirms the antihypertensive effect of quercetin by upregulating the NQO-1 gene and halting oxidative stress. Quercetin also restores altered hemodynamic, biochemical, left ventricular functions, anthropometric and histopathological parameters by retaining antioxidant status. The study also reveals NQO-1 activators to be novel targets for anti-hypertensive treatment.

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