Asian Pac. J. Health Sci., 2016; 3(1):44-52 e-ISSN: 2349-0659, p-ISSN: 2350-0964

Pharmacological and biochemical assessment of Talc on Doxorubicin induced cardiac remodelling in albino Wistar rats

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ABSTRACT

Background and Objectives: Cardiac remodelling may be defined as genome expression, molecular, cellular and interstitial changes that are apparently changes in size, shape and function of the heart after cardiac injury. Type-2 diabetes is associated with significantly higher risk of heart failure (HF), coronary artery disease (CAD) and cardiac remodelling. Present study was aimed as to evaluate the Pharmacological and biochemical effects of Talc in doxorubicin induced cardiac remodelling in Wister rat. **Method:** Albino Wistar Rats (100-150gm) were divided into 8 groups (n=6), cardiac remodelling were induced Doxorubicin with a 2.0 mg/kg i.p. twice in a week for 20 weeks) and oral dose of talc (0.14mg/kg) for the period of 20 weeks and treated with Paracetamol for 5 day.**Result and Conclusion:** Exposure of Doxorubicin and Talc altered all biochemical parameter as Blood glucose, Triglyceride, CRP and Troponin-T etc. In conclusion, it was clear that talc may promotecardiac remodelling in Wistar rats.

Keywords: Blood glucose, Blood glucose, CRP, Troponin-T

Introduction

Remodelling is defined as alteration in the structure (dimensions, mass, shape) of the heart (called cardiac or ventricular remodelling) in response to hemodynamic load and/or cardiac injury in association with neuro-hormonal activation [1, 2]. The cardiac cells involved in the remodelling process are cardiomyocytes and fibroblasts. Fibroblast stimulation increases collagen synthesis and causes fibrosis of both the infarcted and non- infarcted regions of the ventricle [3]. This leads to a loss of cardiomyocytes by apoptosis or necrosis. Eventually these cardiomyocytes are replaced by fibroblasts and extracellular collagen. Doxorubicin (DOX), an anthracycline has been widely used in the treatment of all forms of cancer but its use is limited due to its toxicity effect especially on the cardiac tissues. The Doxorubicin induced cardiotoxicity has been shown to be mediated through different mechanisms, including membrane lipid peroxidation, ROS formation and mitochondrion damage [4]. Due to the lack of developed antioxidant defence system, these free radicals produced by electron transfer from the semiquinone to quinine moieties of the anthracyclinethat are responsible for myocardial damage and subsequent doxorubicin induced cardiotoxicity[5].Paracetamol (known as acetaminophen in the United States) is one of the most commonly used non-steroidal anti-inflammatory drugs (NSAIDs) [6]. It is a rapid, reversible, non-competitive inhibitor of cyclooxygenase activity (Cox-3) in the brain and spinal cord and thus products of the arachidonic acid cascade; acetaminophen has been available since the 1950s as an over-the-counter product for pain and fever relief. Although its metabolism is quite well understood, the mechanism of acetaminophen toxicity remains somewhat a mystery with recent evidence suggesting that multiple cytotoxic pathwayis involved. Paracetamol is a widely used analgesic and antipyretic drug [7].Talc is defined as hydrous magnesium silicate, $Mg_3Si_4O_{10}(OH)_2$ consists of a brucite sheet containing magnesium ions pack between two silica sheets which are held mutually by

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relatively weak forces and belongs to the silicate subclass phyllosilicate and the clay group montmorillonite/smectite. Nickel and iron a variety of elements may be included in the talc particle lattice but are so bound within the particle that they are not free to exert any biological action [8].In the review of pharmaceutical excipients, Golightlyet al.(1988) state, "Ingestion of talc is very unlikely to be toxic[9]."Our previous result was based on the pharmacological action of excipient in anticancer drug, have given an influence that Talc may induced Type-2 diabetes [10]; Diabetes accounted for a significant percentage of patients with a diagnosis of heart failure in numerous epidemiologic studies [11]. The United Kingdom Prospective Diabetes Study [12], Cardiovascular Health Study [13], and Euro Heart Failure Surveys [14] all suggested that the presence of diabetes may independently increase the risk of developing incident heart failure; Thus Cardiac remodelling protocol was designed.Albino Wistar male/female rats weighing about 100-150 gm, were procured from Siddhartha institute of pharmacy, Dehradun. The Albino Wistar rats were housed under temperature (25 ± 2ºC) and relative humidity (30-70%) with a 12:12 light-dark cycle, and acclimatized in the animal house facility of the department under ambient condition. The animals were fed with standard semi purified diet and water. All the experiments on animals were conducted in accordance with the internationally accepted principles for laboratory animal use and as per the experimental protocols duly approved by the Institutional Ethical Committee (IAEC No. **1435/PO/a/11/CPCSEA**) with the project approval no- **"SIP/IAEC/02/2014"** by the Institutional Animal Ethical Committee (IAEC) of Siddhartha College of pharmacy, Dehradun. The rats were acclimatized and randomly divided into 08 groups and each group have 6 rats; **(1)Normal control (NC)** - Rats were administered with normal diet and were not given any treatment during the research study. **(2)DOX Contro**l-Rats of this group were administered Doxorubicin with a 2.0 mg/kg i.p. twice in a week for 20 weeks**(3)Talc control**- Rats of this group were administered with Talc with a 0.14mg/kg oral dose for 20 weeks[10]. **(4)PCM control**- Rats of this group were administered with normal diet for 20 weeks and after that were given PCM (500 mg/kg/d) for 5 days.**(5)DOX +Talc control**- Rats of this group were administered Doxorubicin with a 2.0 mg/kg body weight i.p. twice in a week and also administered Talc with a 0.14mg/kg oral dose for 20 weeks. **(6)DOX +PCM control**- Rats of this group were administered Doxorubicin with a2.0 mg/kg body weighti.p. twice in a week and were given PCM (500 mg/kg/d) for 5 days. **(7)Talc+PCM control**- Rats of this group were administered Talc with a 0.14mg/kg oral dose for 20 weeks and were given PCM (500 mg/kg/d) for 5 days. **(8)DOX+Talc+PCM control**- Rats of this group were administered Doxorubicin with a2.0 mg/kg body weighti.p. twice in a week and administered Talc with a 0.14mg/kg oral dose for 20 weeks and were given PCM (500 mg/kg/d) for 5 days.

Induction of cardiac remodelling

Cardiac remodelling was induced by a Doxorubicin dose of 2.0 mg/kg body weight twice in a week.

Determination of biochemical parameters

Isolation of Serum

At the end of the treatment period, animals were fasted overnight. Blood sample was collected on termination of the experiment from retro-orbital plexus under light ether anesthesia without any anticoagulant and allowed to stand for 30 minutes at room temperature then centrifuged at 250 rpm for 10 minutes to separate the serum. The serum obtained was kept at 2ºC -4ºC until used.

Blood glucose measurement using Glucometer

Blood glucose was measured using digital glucometer. Drop of blood was placed on one side of test strip that was inserted in the glucometer. The glucose level was displayed on to the screen within 20 seconds.

C-reactive protein

The levels of C-reactive protein (CRP) are enhanced by HF. Higher C-reactive protein predicts worse prognosis in acute heart failure only in non infected patients. Recent studies have reported a potential added-value of CRP for the risk prediction of adverse clinical events independently of natriuretic peptides. The potential advantage of CRP testing is its availability within the majority of laboratories through automated assays.

Troponin-T

Cardiac troponin T (cTnT) was measured on Elec-sys 2010 Immunoassay System (Roche Diagnostics, Indianapolis, IN). The method employs two monoclonal antibodies specifically directed against human cardiac troponin T, with electrochemiluminescent detection. This is a recent, sensitive third-generation assay standardized with human recombinant cTNT, with a limit of detection of 0.01 ng/ml. 0.1 ng/ml is recommended as the clinical threshold value, above which damage to the myocardium can be assumed.

Statistical analysis

Statistical analysis was carried out using Graph Pad Prism 5.0 (Graph Pad Software, San Diego, CA, USA).The results were expressed as mean ± SEM.

Statistical significance between more than two groups was tested using one-way ANOVA followed by Tukey's multiple comparison tests. The p values less than 0.001 were considered significant.

Results

Blood Glucose

The DOX control were showed not significant *(ns)* effect and Talc control were displayed significantly increased *(p<0.001)* result of Blood glucose as compared to NC group. On the other hand Talc treated groups displayed significantly elevated level *(p<0.001)* of blood glucose as compared to DOX Control group; while PCM control were showed not significant *(ns)* level of blood glucose when compare with DOX control group**(Table no. 1)**.

Triglyceride

The DOX control were displayed not significant *(ns)* effect and Talc control were displayed significantly upward *(p<0.001)* level of Triglyceride as compared to NC group. On the other hand Talc control and Talc+PCM control were illustrated significantly elevated level *(p<0.001)* of Triglyceride as compared to DOX Control group; while PCM control and DOX+Talc control were showed not significant *(ns)* level of Triglyceride and DOX+Talc+PCM control were demonstrated significant *(p<0.05)* level of Triglyceride when compared to DOX Control group**(Table no. 1)**.

Total Cholesterol (mg/dl)

The DOX control and Talc control were displayed significant *(p<0.001)* level of Total Cholesterolas compared to NC group while Talc control and DOX+Talc control, Talc+PCM control and DOX+Talc+PCM control were displayed significantly increased *(p<0.001)* level of Total Cholesterolas compared to DOX Control. DOX+PCM control were demonstrated not significant *(ns)* level of Total Cholesterolas compared to DOX Control**(Table no. 2)**.

Creatinine (mg/dl)

The DOX control and Talc control were displayed significantly upward *(p<0.001)* level of Creatinineas compared to NC group. Talc control and DOX+Talc control were showed not significant *(ns)* level of Creatinineas compared to DOX Control while PCM control, DOX+PCM control and Talc+PCM control were displayed significant *(p<0.001)* level of Creatinineas compared to DOX Control. In DOX+Talc+PCM control were demonstrated significantly decreased level *(p<0.05)* of Creatininewhen compared to DOX Control**(Table no. 1)**.

HDL (mg/dl)

The DOX control and Talc control were displayed significantly downward *(p<0.001)* level of HDL as compared to NC group; while Talc control and DOX+Talc control were showed not significant *(ns)* level when compared with DOX control. In Talc+PCM control and DOX+Talc+PCM control were demonstrated significant level *(p<0.001)* level of HDL as related with DOX control**(Table no. 2)**.

LDL (mg/dl)

The DOX control were displayed significantly upward *(p<0.001)* level of LDL and Talc control were showed not significant *(ns)* level of LDL when compared with NC; on the other hand if Talc control and DOX+Talc control were compared with DOX control it showed not significant *(ns)* level of LDL. DOX+Talc+PCM control were demonstrated significant level *(p<0.001)* level of LDL as compared with DOX control**(Table no. 2)**.

Cal^{2+} ion (m.eq/L)

The DOX control and Talc control were displayed significantly downward *(p<0.001)* level of Calcium ion as compared to NC group; while if Talc control, PCM control, DOX+Talc control and Talc+PCM control were showed not significant *(ns)* level of Calcium ion when compared to DOX Control. In DOX+Talc control and DOX+Talc+PCM control were demonstrated significantly increased level *(p<0.001)* of Calcium ion when compared to DOX Control**(Table no. 2)**.

Na^{+} ion(m.eq/L)

The DOX control and Talc control were illustrated not significant *(ns)* level of Sodium ion as compared to NC group; while if Talc control, PCM control, DOX+Talc control, Talc+PCM control, DOX+Talc and DOX+Talc+PCM were also displayed not significant *(ns)* level of Sodium ion when compared to DOX Control**(Table no. 2)**.

K^{+} ion (m.eq/L)

The DOX control were demonstrated not significant *(ns)* level and Talc control were displayed significantly upward *(p<0.001)* level of Potassium ion as compared to NC group; while if Talc control, DOX+Talc control and Talc+PCM control were compared to DOX Control it illustrated significantly increased *(p<0.001)* level of Potassium ion. In PCM control and DOX+PCM control were showed not significant *(ns)* level and DOX+Talc+PCM control were demonstrated significantly increased level *(p<0.05)* of Potassium ion when compared to DOX Control**(Table no. 2)**.

CPK-MB (U/L)

The DOX control Talc control were displayed significantly upward *(p<0.001)* level of CPK-MB as compared to NC group; while if Talc control, PCM control, DOX+Talc control, Talc+PCM control and DOX+Talc+PCM were compared to DOX Control it

illustrated significant *(p<0.001)* level of CPK-MB. In and DOX+PCM control were demonstrated significantly decreased level *(p<0.01)* of CPK-MB when compared to DOX Control**(Table no. 1)**.

CRP (mg/L)

The DOX control and Talc control were displayed significantly increased *(p<0.001)* level of CRP as compared to NC group; while if Talc control, DOX+Talc control were compared to DOX Control it illustrated not significant *(ns)* level of CRP. PCM control and Talc+PCM control were significantly decreased *(p<0.001)* level of CRP when compared to DOX Control while In DOX+Talc+PCM control were demonstrated significantly diminished level *(p<0.01)* of CRP when compared to DOX Control**(Table no. 1)**.

Troponin-T (U/L)

The DOX control displayed significantly increased *(p<0.05)* level of Troponin-T and Talc control were displayed significantly increased *(p<0.001)* level of Troponin-Tas compared to NC group; while if Talc control, DOX+Talc control, Talc+PCM control and DOX+Talc+PCM control were compared to DOX Control it illustrated significantly elevated *(p<0.001)* level of Troponin-T when compared to DOX Control**(Table no. 1)**.

Table 1:Data showing the effect of Talc on the levels of Blood glucose (mg/dl), Triglyceride (mg/dl), Creatinine (mg/dl), CRP (mg/L), Troponin-T (U/L), CPK-MB (U/L) of wistar rats

S.No.	Groups	Blood glucose (mg/dl)	Triglyceride (mg/dl)	Creatinine (mg/dl)	CRP (mg/L)	Troponin-T (U/L)	CPK-MB (U/L)
1	Normal control (NC)	73.83±3.439	47.67±2.418	0.620±0.0404	1.733±0.0666	0.533±0.0196	65.83±4.729
2	DOX control	83.50±1.565^{ns}	56.33±1.838^{ns}	0.988±0.0403$^{###}$	3.083±0.1249$^{###}$	1.212±0.2056$^{#}$	295.0±12.78$^{###}$
3	Talc control	240.7±4.631***	122.7±2.985***	0.978±0.0577^{ns}	2.650±0.1478^{ns}	4.700±0.1713***	614.7±3.575***
4	PCM control	95.83±2.937^{ns}	61.83±1.815^{ns}	0.650±0.0483***	1.733±0.0666***	0.7667±0.0971^{ns}	173.8±2.496***
5	DOX +Talc control	213.3±9.972***	60.83±2.286^{ns}	1.022±0.0304^{ns}	2.850±0.1335^{ns}	3.683±0.1621***	198.3±12.49***
6	DOX +Met control	93.67±3.748^{ns}	69.00±2.745*	0.681±0.0094***	2.517±0.1352*	1.075±0.1624^{ns}	208.7±30.80**
7	Talc+ PCM control	233.3±6.280***	80.00±3.152***	0.723±0.0283***	2.250±0.1176***	2.983±0.1400***	200.8±12.81***
8	DOX+Talc+PCM control	225.0±3.651***	68.33±1.022*	0.815±0.0166*	2.367±0.1430**	2.283±0.1138***	129.0±7.234***

Values are expressed as mean ± SEM (N= 6). ($^{#}$) Groups as compared to normal control, (*) Groups as compared to DOX control. *ns –no significant; * P<0.05; **P<0.01; ***P<0.001.*

Table 2: Data showing the effect of Talc on the levels of Total Cholesterol (mg/dl), HDL (mg/dl), LDL (mg/dl), Cal^{2+}ion (m.eq/L), Na^{+}ion (m.eq/L), K^{+}ion (m.eq/L) of wistar rats

S.No.	Groups	Total Cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Cal^{2+}ion (m.eq/L)	Na^{+}ion (m.eq/L)	K^{+}ion (m.eq/L)
1	Normal control (NC)	72.00±3.642	21.67±0.8819	45.0±1.713	9.233±0.0666	151.0±0.730	4.050±0.0763
2	DOX control	127.5±2.141$^{###}$	12.83±0.6009$^{###}$	59.17±1.956$^{###}$	8.10±0.243$^{###}$	152.5±0.7638^{ns}	4.383±0.1869^{ns}
3	Talc control	110.8±3.070*	14.33±1.022^{ns}	52.67±1.606^{ns}	7.667±0.156^{ns}	148.5±1.607^{ns}	6.383±0.233***
4	PCM control	71.50±1.875***	23.00±1.155**	43.67±1.476***	7.633±0.088^{ns}	152.5±0.763^{ns}	4.683±0.0749^{ns}

5	DOX +Talc control	105.8±2.301 **	11.67±0.5578 ns	59.67±1.926 ns	9.100±0.1065 ***	150.7±0.666 ns	6.617±0.1922 ***
6	DOX +Met control	96.67±6.280 ***	18.17±0.7923 **	47.50±2.487 ***	8.650±0.0763 ns	151.3±1.145 ns	4.733±0.111 ns
7	Talc+ PCM control	93.17±2.12 * **	23.33±0.8819 ***	48.33±1.726 **	7.750±0.125 ns	150.7±1.430 ns	6.467±0.154 * **
8	DOX+Talc+PCM control	105.0±4.830 **	19.50±0.4282 ***	40.67±1.116 ***	9.050±0.0428 ***	152.8±0.945 ns	5.262±0.241 *

Values are expressed as mean ± SEM (N= 6). (#) Groups as compared to normal control, (*) Groups as compared to DOX control. *ns –no significant; * P<0.05; **P<0.01; ***P<0.001*.

Discussion

Cardiac remodelling is defined as genome expression, molecular, cellular, and interstitial changes that manifest clinically as changes in the size, shape, and function of the heart after cardiac injury [1] or increased cardiac load.Type 2 diabetes is associated with significantly higher risk of heart failure (HF), coronary artery disease (CAD) and cardiac remodelling [15].The current American Heart Association, heart failure classification schema designates the presence of diabetes mellitus as stage a heart failure, which raises the risk of developing stage B heart failure or asymptomatic left ventricular (LV) dysfunction [16]. Individuals with diabetes frequently have LV remodelling; both increased LV mass and dilatation have been reported [17]; Researchers have also observed that factors associated with insulin resistance syndrome predate the development of LV systolic dysfunction by 2 decades, adjusting for ischemic heart disease and other risk factors [18].Doxorubicin is an anti-tumour drug that is useful in treating several types of cancer, although its clinical use has been restricted due to cardiomyopathy induced by dose-dependent cardiotoxicity. Once cardiomyopathy occurs, treatment options are few, and doxorubicin-induced heart failure is usually refractory to conventional therapy. Hence, to evaluate Talc effect on doxorubicin-induced cardiomyopathy is the goal [19]. In the present study DOX control rats were not increased the level of glucose nor triglyceride, on the other hand Talc treated rats were demonstrated the significantly *(p<0.001)* high blood glucose level, altered insulin level, significant *(p<0.001)* higher circulating levels of triglycerides and lower high-density lipoprotein (HDL)-cholesterol concentrations and commonly manifest the metabolic syndrome [20]. The metabolic syndrome is a predictor of future risk of cardiovascular disease[21]. Specifically, it is widely recognized that higher levels of small dense low-density lipoprotein particles are a major contributor of increased risk for cardiovascular disease in insulin-resistant patients [22]. The association of different lipid fractions (especially the total cholesterol to HDL-cholesterol ratio) with incident heart failure is not the same as that for incident atherosclerotic cardiovascular events [23]. Evidence supports that higher triglyceride levels [24] or higher non-HDL–cholesterol levels [25]increase the risk of developing heart failure, perhaps because of the presence of insulin resistance that predisposes to metabolic changes characterized by impaired myocardial fatty acid oxidation and greater uncoupling of mitochondrial proteins in the heart [26]. Therefore in the Talc treated rats an insulin-resistant condition is developed which decreased uptake of myocardial glucose [27], resultant myocardial cells incapable to metabolize pyruvate, so that lipid accumulation in the myocardium is started [28]. However, the mechanisms by which free fatty acid metabolism and glucose oxidation are interrelated are quite complex [29]. Increased activation of different isomers of protein kinase C has been noted to play a role in inhibition of insulin secretion by free fatty acids [30]. On the other hand, hyperinsulinemia in diabetic patients is also associated with increased free fatty acid levels, elevated heart rate, and increased activation of sympathetic nervous system; all these factors subsequently lead to cardiac hypertrophy and intracellular accumulation of triglycerides. In the presence of hyperinsulinemia and insulin resistance, the heart has also been shown to reduce protein degradation, which may then promote myocardial hypertrophy [31]. In addition, dyslipidemia and production of free radicals result in alteration of genetic transcription factors and coding, which subsequently alters the translation of nucleoprotein genes such as those in the renin-angiotensin system (RAS) and IGF-1. Increased expression of RAS genes promotes insulin resistance, whereas greater expression of the IGF-1 gene increases sensitivity of cardiac myocytes to calcium concentrations and increases myocardial contractility. IGF-1 also acts synergistically

with angiotensin II in promoting cellular and myocardial hypertrophy [32].Doxorubicin intoxication caused a significant increase *(p<0.001)* in the plasma concentration of Cholesterol, LDL and triglycerides and reduction in HDL; similar result was illustrated by Talc treated wistar rats. This indicates that doxorubicin and Talc may be interfering with metabolism or biosynthesis of lipids. Alterations in lipid metabolism and serum levels of cholesterol, triglyceride and LDL are some of the biochemical indices that are often used in the prediction of cardiovascular disease risk [33]. Diabetes mellitus is frequently associated with impaired lipid metabolism such as elevated cholesterol and triglycerides (TG) and abnormalities in serum lipoproteins [34]. Talc treated rats showed elevation of blood glucose level leads disturbance in triglyceride level of blood which further interfere with cholesterol, lipid metabolism, lowered HDL and increased LDL level; that could generate cardiac dysfunction in Wistar rats and by the time may induces cardiac remodelling like condition. A high level of LDL can be suggestive of medical problems such as diseases of cardiovascular system, also the higher the level, the higher the risk for coronary artery disease in Talc treated rats [35, 36]. Lipids play an important role in the pathogenesis of myocardial ischemia. Hypercholesterolemia and hypertriglyceridemia are the risk factor for the development of myocardial ischemia. Increased levels of blood cholesterol and their accumulations in the heart are well associated with myocardial damage [37].Creatinine is filtered out of the blood by the kidney and is a direct measure of renal excretory function. If the filtering by the kidney is deficient, plasma levels rises above normal, the urine levels reduces. Serum creatinine concentration increases when more than 50% of renal function has been lost [38]. Although creatinine, like urea, is not generally considered to be an important uremic toxin, both compounds have been shown experimentally to be toxic in acutely uremic rats [39].It is however a known fact that kidney function is compromised in uncontrolled diabetes mellitus. Glycosuria, which is a pertinent diagnostic feature of diabetes, causes dehydration via glucose osmotic diuresis. This dehydration is accompanied with severe loss of electrolytes including sodium, potassium, calcium, chloride and phosphates. A decrease in concentration of plasma sodium (hyponatremia) could be suggestive of diseases of the liver, kidney or congestive heart failure [40]. Persistent low levels of sodium in plasma in diabetic rats here is unacceptable because of the increased risk of developing congestive heart failure. In the present study plasma sodium level were slightly decreased in Talc control, but on comparison with normal control and DOX control it were not significant *(ns)*. Potassium depletion inhibits insulin secretion and is associated with glucose intolerance, whereas potassium infusion and hyperkalemia increase the secretary rate of insulin by changing the membrane potential of pancreatic beta cells [41]. In the current protocol Talc control showed the significant increased level of plasma Potassium level which indicated that Talc treated Wistar rats trying to maintain the insulin level by changing potential of beta cells.

In addition, Ca^{2+}handling alterations in vascular smooth muscle cell (VSMCs) contribute to blood vessel dysfunction associated with diabetes. VSMCs provide not only structural integrity to the vessels but also actively regulate arterial tone and local blood pressure via diverse Ca^{2+} signalling pathways [42]. Vascular dysfunction is a distinctive phenotype found in both types of diabetes and likely contributes to the high incidence of stroke and heart attack, not to mention organ damage, such as retinopathy and nephropathy, that is observed in diabetic patients [43, 44], The CK system of isoenzymes consists of CK-MM, CK-MB, CK-BB, and mitochondrial-CK. Each subunit of the dimeric CK is regulated by a distinct gene, and expressed in a tissue-specific manner. In humans and animals, CK-MB is found predominately in the myocardium; with concentration ranges from 5 to 30% of the total CK activity of the heart. The largest portion of the heart is composed of CK-MM. CK-MB, a golden standard marker of myocyte injury or death, leaks out from myocardium due to disintegration of the contractile apparatus and increased sarcoplasmic permeability. When ischemia destructs cell membrane, these enzymes are leaked out of cells[45]; so the level shows the injury rate and cell necrosis [46]. In Talc control and DOX control significantly *(p<0.001)* elevated level of CK-MB clearly said that Talc may responsible for the myocardial cell injury or membrane integrity in current research. C-reactive protein (CRP) as a sensitive marker of inflammation has been widely studied, its level regulated primarily by the function of cytokines, such as interleukin-6 (IL-6). It binds to a wide variety of substances, such as microbial polysaccharide, phosphatidylcholine, and damaged cell membrane. CRP also enhances the activity of phagocytic cells and activates the classical complement pathway [47]. The differences seen in CRP concentrations between the normal control, DOX control and Talc controls remained highly significant after the adjustment for hypertension, diabetes. These increases in CRP levels also reflect the severity of coronary disease [48]. Our data of the elevated CRP levels in the wistar rats with increased levels of LDL may raise the possibility of the cardiac disorder.

Cardiac troponin is regulatory proteins that control the calcium mediated interaction of actin and myosin resulting in contraction and relaxation of striated muscle. Greaser et al. demonstrated that the troponin complex comprised three distinct proteins: troponin C (TnC), for binding $Ca2^{+}$ and regulating thin filament activation; troponin I (TnI), for inhibiting actin-activated myosin ATPase activity; and troponin T (TnT), for binding tropomyosin (Tm) [49]. Troponin I and T are two proteins of the thin filament regulatory system of the contractile complex of heart and skeletal muscle. TnI is encode by three different genes that are differentially expressed by various muscle tissues. Cardiac troponin I (cTnI) is uniquely specific for the heart, containing a 31-amino acid sequence on its N-terminus that differentiates it from the fast and slow skeletal forms. Troponin T is also expressed by three different genes, resulting in slow and fast skeletal isoforms and a cardiac (cTnT) form [50, 51]. Cardiac troponin T is an established biochemical marker of myocyte damage which can be used to assess myocyte damage from myocardial infarction [52].Increased TnT in the circulation translates to heart injury. Troponin predicts the occurrence of clinically significant LV dysfunction at least 3 months in advance [53, 54]. The observation that Troponin T concentrations are significantly increased *($p<0.001$)* in the DOX and Talc treated wistar rats, which clearly indicated that may some injury happened in myocardial wall, previous researcher gave us various case study that supported protocol results.Cardiac troponin plays a crucial role in the diagnosis of myocardial necrosis. According to the universally accepted definition, myocardial infarction is diagnosed when blood levels of sensitive and specific biomarkers such as cTn or CK-MB are increased in the clinical setting of acute myocardial ischemia [55-56]. Although cTnT and cTnI increase in blood reflect injury leading to necrosis of myocardial cells, they do not provide any clues with respect to the underlying disease mechanism. Various possibilities have been suggested for the release of structural proteins from the myocardium, including normal turnover of myocardial cells, apoptosis, cellular `release of troponin degradation products, increased cellular wall permeability, formation, and release of membranous blebs and myocyte necrosis.

Conclusion

In the conclusion, it can be say that due to increased plasma glucose and Triglyceride level in the blood, insulin resistance may occur; it mayalteredthe lipid metabolism which generated the ROS production in heart wall and deposited fatty acid in blood vessels wall this may changes the blood flow; therefore blood pressure is increased and activation of rennin angiotensin system occur, may deposition of fatty acid damage the blood vessel wall.By altering Troponin T, CRP; Talc may stimulate the vascular inflammation by various circulating proinflammatory cytokines such as interleukin-6, interleukin 1-beta. These cytokines are responsible to generate the NOS expression and nitrite production which produce ROS formation and subsequent MAPK development may lead oxidation of myofilaments and induced cardio-myocyte apoptosis; furthermorethis may promote the diabetes cardiomyopathy and if this condition retain for long time it will lead cardiac remodelling,but through intensive research is required for our understanding of the role of Talc as a promoter of cardiac remodelling, it represent attractive but challenging targets for the future.

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Source of Support: Nil
Conflict of Interest: None