6. Discussion:

The insufficiency in the cardiac output and blood flow of the heart to meet the needs of the body is defined as a heart-failure (Burton & William, 1972). The cardiovascular risk factors and its pathogenesis don't depend on the gender in a wider form. But male and female hormonal differences, family history and smoking habits raise an impact on the cardiovascular disease outcome. In rat experiment, high fat diet increased serum cholesterol and triglyceride. A long term dyslipidemia has been demonstrated in the epidemiological data and that can predict an atherosclerosis and coronary artery syndrome (Soler & Ruiz, 2010). Moreover, abundance increase in free radical in liver tissues in the experimental rat in the current experiment may have some added effects on cardiac pathology. Increase in MDA without a possible protection from soluble thiol make this tissue more vulnerable and consequently increase the systemic stress in the rats of high lipid-fed group. Both male and female cardiac patients are shown with appreciable higher level (53%; $p<0.05$, 56%, respectively) of lipid peroxidation with lesser protection from antioxidant thiol (-SH) in the current study. The higher level of oxidative stress in dyslipidemic condition in our animal study may be correlated with our human experiment results.

In the current study, no significant differences were noticed when the values were compared between male and female except the serum LDL level in control. Taken into account the fact that inter-individual variability is very much likely in a randomly selected sample distribution, it is more general that the termination effects of estrogenic function may manifest more definitive consequences. Significant fatal changes in most of the risk factors/cardiac markers in trop T groups (positive or negative) are even more than that of male individuals (fig-3). This shows that female are more sensitive and considering their age

group, post menopausal condition is likely to be one of the important determinant. Several studies have shown that more than one fourth of men is affected with a chronic heart-failure are also deficient in some steroids i.e. testosterone (Naghi et al., 2011). Conversely, estrogens have a cardio-protective effect via multiple mechanisms. And postmenopausal women are noticed to be in a greater chance of risk of several metabolic disorder including diabetes and cardiovascular disorders. A mechanism of estrogen has been shown to increase expression of superoxide dismutase (SOD) and inhibition of NADPH oxidase activity. As a result it reduces oxidative stress in the female (Lagranha et al., 2010; Xing et al., 2009; Zhang & Wang, 2009). In the current study the higher SOD activity is shown both in male and female cardiac patients as demonstrated in the NBT reduction gel zymography assay (fig 9). Beside oxidative stress, inflammation is considered to be an important component in the pathogenesis of hypertension, atherosclerosis and the development of coronary heart disease (CHD). Estrogen has been reported to reduce inflammatory responses (Xing et al., 2009; Reckelhoff, 2006).

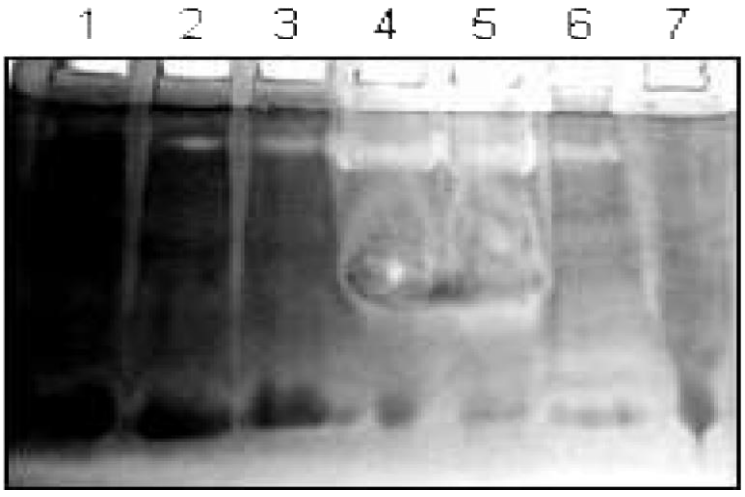

Fig-9. Superoxide dismutase activity in human serum. Lane distribution: serum SOD activity of cardiac markers and cardiac risk factors comparison to the control; lane-1&2 control, lane-3 high triglyceride (Tg), lane-4 & 5 Trop-t_{+ve} ,lane-6 & 7 high Tg and cholesterol (Cho).

In women, other cardio-protective mechanisms of estrogens are the increase of high-density lipoprotein (HDL), decrease of low-density lipoprotein (LDL), and release of vasodilators such as nitric oxide (NO) and prostacyclin (PGI2) from vessel walls. That results in the inhibition of vascular constriction and lowering of blood pressure as well as platelet aggregation (Barrett-Connor & Bush, 1991). Nitric oxide which can be generated by estrogen and insulin is produced in response to various stimuli. These including fluid shear, stress, and exposure to neurohumoral factors such as acetylcholine, bradykinin, serotonin, and substance P. Several of which are shown to be enhanced in prior or during cardiovascular pathogenesis. The NO is a potent vasodilator that contributes to the maintenance of the basal vascular tone and blood flow and thus to the physiological regulation of blood pressure (Vallance et al., 1989). It is well established that the occurrence of heart failure after menopause is due to a low level of circulating estrogen (E2). Cytoprotective role of E2 is the important feature that functions in different mode verified other physiological factors a likely to contribute that. The ovarian synthesis of the estrogen experiences a sharp decline after the menopause but its intra-myocardial synthesis is less influenced by such physiological variations (Grohe et al., 1998).

Whether estrogen alone is cardioprotective in humans needs to be clarified. Indeed, the observed gender differences and the increased incidence of cardiac disorder among women after menopause might not entirely be due to the reduction of estrogen, but might be associated to the testosterone. It is known that the post-menopausal ovaries secrets significant amount of androgens in the form of testosterone and androstenedione (Sluijmer et al., 1995). A positive correlation has been illustrated in the post-menopausal women between increased

testosterone levels and hypertension, decreased HDL, destructed vascular reactivity, cardiac hypertrophy, and coronary artery disease (Phillips et al., 1997). This is apparent to say that during postmenopausal state female hormonal cum metabolic regulations run more in male like fashions. This particular situation, in addition to the estrogenic cessation exerts a compounding effect on the female metabolic systems. In case of female severity in disease factor or the stress condition is little more. One important thing that these individuals were more than 45 years age group. At this age females experience post-menopausal condition. This may be one of the causes. For the first time, in a mouse model of myocardial infarction (MI) (Cavasin et al., 2003) postulated that estrogen and testosterone play variant and opposing roles in the development of heart failure and long-term remodeling after MI. In particular, estrogens (either endogenous or supplemental) defend maladaptive chronic remodeling and further deterioration of cardiac pathogenesis, whereas testosterone (either endogenous or supplemental) adversely affects myocardial healing (as indicated by a higher rate of cardiac events), supports cardiac dysfunction and remodeling, and exerts disease pathogenesis when estrogen levels are reduced.

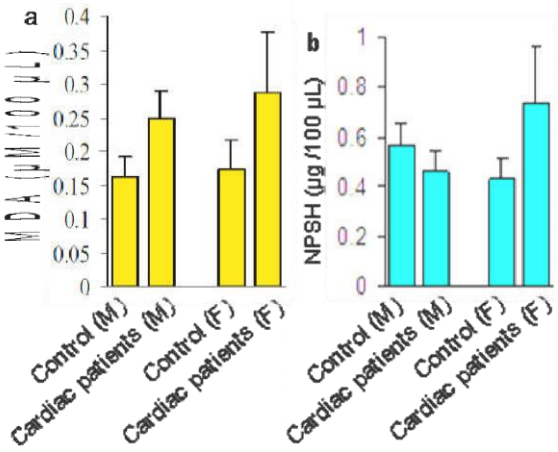

Fig-10. Bar diagram shows co parison of MDA (a) and NPSH (b) level in blood serum of male & female cardiac patients with the corresponding control group. Sample sizes- male control; 10, male cardiac patients 11, female control- 12, female cardiac patients-10. The values are denoted as mean ± SE.

It may be one of the explanations for the male cardiovascular failure. However, it should be observed that plasma estrogen and testosterone levels in males and females who received hormone replacement therapy were much greater than the physiological levels. Super oxide dismutase (SOD) plays a vital role in the controlling of the mitochondrial reactive oxygen species (ROS) produced during normal oxidative phosphorylation and defends cells against oxidative stress by catalytic removal of superoxide anion radicals and conversion to hydrogen peroxide (Dhalla et al., 2000). In the present study, the rat experiments suggest that feeding of high lipid containing diet and eventually the dyslipidemic states have significant effects on SOD and catalase activity in the liver and heart tissues. High lipid or fructose feeding for 90 days significantly increased SOD and catalase activities. Myocardium showed enhanced susceptibility to lipid peroxidation, a type of oxidative

degeneration of polyunsaturated fatty acids which is recognized as one of the basic deteriorative reactions during myocardial ischemia (Stanely Mainzen Prince et al., 2011). A study demonstrated when isoproterenol (ISP) is treated to rats; the myocardial cells containing CPK-MB isoenzyme and LDH are damaged or destroyed due to a reduction of oxygen or glucose. The integrity of the cell membrane becomes disordered and it might become more porous/ permeable or may rupture. This type of deregulation and cellular damage result in leakage and increase of several enzymes/ molecules (i.e. SGOT, CPK-MB, Trop-I and LDH) in the circulation. Serum level of cardiac Trop-I is closely associated with the degree of myocardial injury (Bertinchant et al., 2000; Wallace et al., 2004). The ISP increased in the level of inflammatory markers viz. IL-6, C-reactive protein (CRP) and tumor necrosis factor α (TNF-α), which mainly evolve in the serum during inflammation as evident during ISP challenge. The intracellular changes occurred in due course of ischemic injury such as accumulation of H+ and Ca2+ as well as the disruption of mitochondrial membrane potential, leading to the formation of oxygen free radical or ROS. The ROS accumulation and the subsequent activation of pro-inflammatory pathways play an important role in the ischemic injury. Therefore, a crucial mediator of ischemic injury is excessively generated by derived free radicals, leading to different forms of oxygen species (Farvin et al., 2006). Reactive oxygen intermediates directly damage to cellular macromolicules i.e. DNA, protein, and lipids, in addition to activating pathways of stress response. This nonspecific injury promotes a cytokine-mediated cascade reaction, which induced the production of tumor necrosis factor alpha (TNF-α) (Kumar et al., 2009). Finally, oxidative stress-induced damages of the cardiac tissue are further promoted by the enhanced inflammatory responses and

cardio-vascular anomalies. Though it seems less variations in male and female values between corresponding groups but very high inter-individual variability especially in case of SGOT, LDH, CPK and CPK-MB minimize the statistical strength to be significant. But it may be hypothesized from the present investigation that the proposed markers and risk factors are significant for the disease initiation and pathogenesis both in male and female. The females of the present age group are more sensitive responded distinctly in terms of the present metabolic profile/parameters. To make concluding remark large number of individual study is necessary. The present investigation has been done in a specific experimental/clinical settings in a socio-economically, nutritionally alike individual groups.

It is known that over nutrition, obesity, high calorie intake associated with diabetic condition. This is illustrated from population study as well as from laboratory experimental animal. Further this diabetic condition has also been associated with cardiac disorder or pre-cardiac pathogenic condition. This is clearly demonstrated in this study also shown for previous study report on as large number of epidemiological investigation. One interesting report suggests that not only over nutrition but also under nutrition chronically low calorie intake may also produce severe hyperglycemic condition. And this chronic hyperglycemic state may also promoted type-II diabetes. This is also suggest that long term diabetes condition whatever it is caused by, may also initiate other metabolic disorder by similar manner as resulted from over nutrition associated diabetes. So both the population under nutrition as well as over nutrition is under the threat of cardiovascular syndrome, renal disorder or cerebral stroke.

The increasingly number of individuals among the young age group diagnosed as diabetes mellitus in developing countries results in prolonged exposure to gluco-lipotoxicity, sub-threshold inflammation and increased oxidative stress, which put enormous strain on pancreatic β-cells (Kong et al., 2013). In another project in our laboratory, it has been demonstrated that most of the diabetic patients irrespective to their nutritional status, show lower level of serum non-protein soluble thiol (NPSH) with higher level of oxidative-stress marker malondialdehyde and atherosclerotic lipid component (cholesterol and triglyceride). This suggests that impaired glucose homeostasis significantly influence fat metabolism. The precise mechanism by which obesity leads to insulin resistance and to T2DM is not completely known but it may be related to several bio-molecules such as abnormalities in free fatty acids, adipokines, leptin and other substances (Ginter & Simko, 2012).

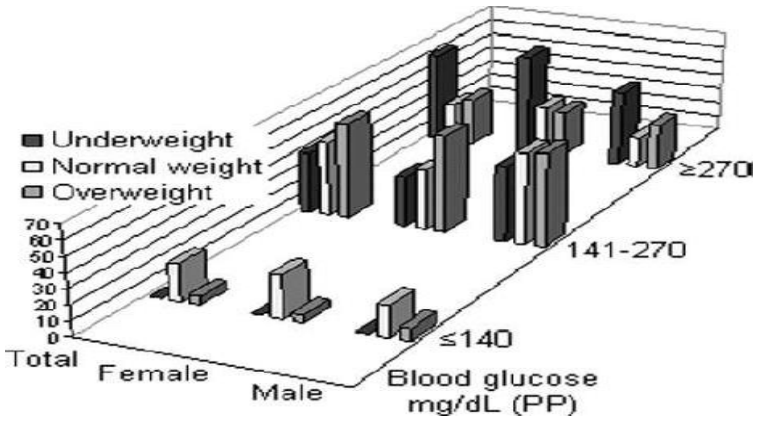

Fig-11. Nutritional status wise distribution patterns (%) of individuals from different groups of blood glucose level.

One recent hypothesis from our research group has explained the fact of the translocation of glucose transporter-4 (GLUT-4) in the production of insulin in the hepatic tissues. And this translocation and synthesis mechanism has been shown to be up-regulated

by the glucose induced activation of hepatocyte membrane nitric oxide synthase (NOS) (Bhattacharya et al., 2013). In case of fatty liver or other impairments in this organ, insulin resistance and hyperglycemia may develop. This finding may suggest the role of extra-pancreatic insulin in glucose homeostasis. A certain degree of impaired liver function may develop insulin resistance. A report reveal that adiponectin gene variants and haplotype contribute to the genetic risk towards the development of type 2 diabetes, obesity and hypo-diponectinemia, which has been demonstrated in a large number of population (Ramya et al., 2013).

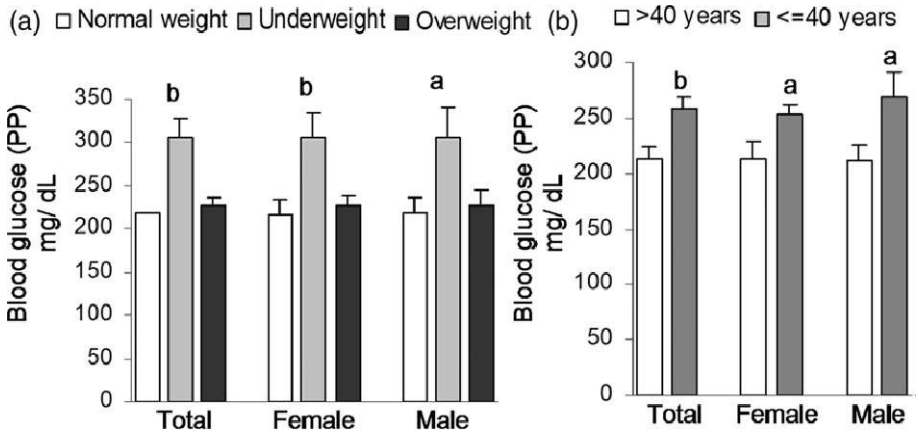

Fig-12. Comparison by Student's t test of underweight and overweight blood glucose levels with that of the participants of normal body weight group (a). Age wise comparison of blood glucose levels between >40 years and ≤40 years group (b). Bar represents the mean ± SE. Level of significance is denoted as a=P< 0.05 and b= P< 0.01

A report reveals that the increase in some allele in malnutrition-related diabetes (MRDM) patients, and the immunogenetic basis, result in certain associations between inflammation and malnutrition in their diabetic manifestations in eastern India (Sanjeevi et al., 2002). The MRDM patients are typically young at onset with low body mass index and

with an insulin-resistance (Kanungo et al., 2002). These reports are in agreement with our findings that unlike normal or overweight group, all malnourished individuals occupy 141–270 and ≥270 mg/dL glucose groups and those are mainly from ≤40 year age group. However, MRDM may co-exist with insulin dependent diabetes mellitus (IDDM) in these patients and that malnutrition could be one of the reasons for the slower onset in IDDM-affected individuals (Sanjeevi et al., 2002). The influence of HLA class II gene polymorphism in MRDM patients is also evident in Eastern India (Sanjeevi et al., 1999). Endothelial dysfunction in malnourished individuals and a stronger influence of obesity on blood pressure have been suggested (Stanner et al., 1997). Anti-oxidant status, oxidative stress and DNA damage in the aetiology of malnutrition related diabetes mellitus have been proposed (McDonagh et al., 1997). A trend of higher blood glucose level (> 270 mm/dl) is noticed among underweight individuals (60%) in our present study. In the perspective of health and nutritional awareness, evidence of ingestion of cyanogens and in others consumption of coarse cereals containing some pancreatotoxins and hepatotoxins are noteworthy. It is important to recognize the etiogenesis of the entity as this may provide preventive public health measures. As discussed earlier, hepatic damage may also result in impairment of energy metabolism, insulin resistance, fatty liver and other metabolic syndrome. Malnutrition merely predisposes the islet cell to some diabetogenic agents that damages pancreatic endogenous and exogenous function to varying degree. If this agent/s can be identified, preventive measures can be evolved. One interesting findings we noticed that a significant number of underweight individuals both in male and female show higher percent

of severe diabetes condition. As because these this data was collected from rural low socio-economic individuals. It can be suggested that under weight individuals are also sensitive to diabetic condition.

The present results demonstrate that stress induced protein DCN-2, plays a critical role to propagate the risk in acute myocardial infarction (AMI). It also induces the cytokine level particularly of TNF-α and IL-6 which causes an inflammatory response after atherosclerotic plaque rupture.

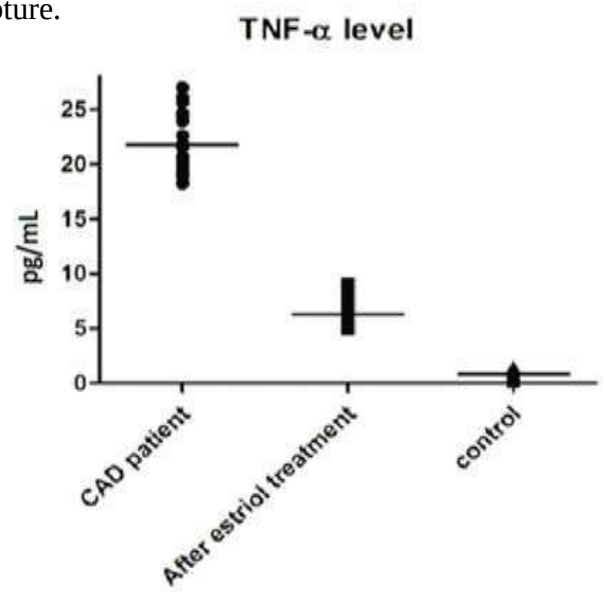

Fig-13. Estriol effect to inhibit TNF-α level in patients with coronary artery disease (CAD) neutrophils. Neutrophils isolated from CAD patients' blood (n=9, M=7, F=2) were subjected for the determination of TNF-α (median value 21.863 pg/ml) as denoted by solid circle (-●-). The level has been found to be decreased (median value 7.43 pg/ml) due to incubation of 0.6 nM estriol as described in solid square (-■-). Solid triangles (-▲-) describe the TNF-α level in age and sex matched controls of the patients with median value 1.14 pg/ml.

It has been illustrated by other researchers that incubation of TNF-α to cell suspension in vitro inspire membrane bound cell adhesion proteins to be activated (Hauser et al., 1997)

and causes aggregation between cells. In case of platelet aggregation which occurs as the post-atherosclerotic plaque rupture phenomenon, the platelets got activated cytokines and those helps to form inter-platelet fibrin mass from activated fibrinogen (Maione et al., 2011). Though aggregation of platelets acts as a lifesaving process but occurrence of this phenomenon at the wrong place causes the loss of life. As other investigators reported before that dermcidin-2 (DCN-2) plays a role on platelet aggregation (Ghosh et al., 2011) like other platelet aggregating agents i.e. ADP, l-epinephrine, thrombin and collagen, our results describe that the promotion of DCN-2 induced platelet aggregation and its enhanced severity occurs through the elevation of cytokine level like TNF-α and IL-6.

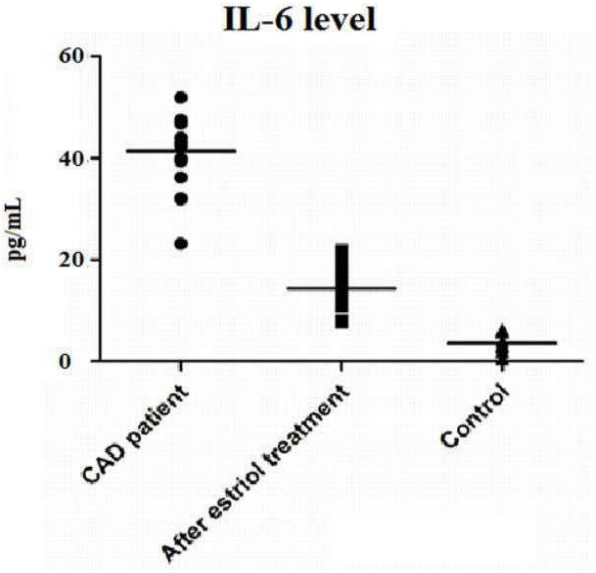

Fig-14. Role of estriol on IL-6 level in CAD patients. IL-6 was determined in same CAD patient neutrophils by ELISA method. IL-6 level in patient group were described as solid circle (-●-) and has the median value with 42.163 pg/ml. The solid squares (-■-) show IL-6 level in CAD neutrophils after 0.6 nM estriol treatment (median value 4.433 pg/ml). Age and sex matched controls were determined for IL-6 level, described in the solid triangles (-▲-) with median value 4.76 pg/ml.

In our study estriol has been shown to induce strong anti-inflammatory responses by inhibiting TNF-α and IL-6 production in neutrophil. There are some reports on neutrophils regarding glucose metabolism, CAD. Neutrophil is the highest percent constituent of blood

cell (70%). So its genomic content and high amount of surface area may also influence in several metabolic process. So we noticed that neutrophil has a significant role in inflammatory marker regulation. Since the contributing volume of this leukocyte in blood is large so its effect could have been immense. And DCN-2 has been shown to induce inflammatory events. It has been reported earliar that the platelet membrane have a sufficient number of TNF receptor (TNF-R) and help to activate platelets (Limb et al., 1999). Particularly estrogens, female steroid hormone have a protective role on cardiovascular disease during child bearing ages (Nelson et al., 2002; Gouva & Tsatsoulis, 2004). It has also been found that estriol like aspirin induces NO synthesis and inhibits DCN-2 induced cytokine synthesis. This phenomenon infers that synthesis of NO could inhibit the expression of inflammatory cytokines. From the present results it has been found that the pre-treatment of estriol to the normal neutrophil solution affects more on the DCN- 2 induced cytokine synthesis while the addition of estriol after DCN-2 treatment has shown a diminished effect. This demonstrates that a preliminary upper level of estrogen like molecules in the system may perform a protective role on stress and cytokine induced platelet aggregation but the pre-occurrence of higher level DCN-2 could repress the protective role of estrogen. It was found that estriol has the ability to partially decrease the level of the cytokine as TNF-α and IL-6. But, IL-6 level was not diminished even after estriol treatment, when neutrophil solution was incubated with 120 nM DCN-2. This signifies a serious prognosis of outcome in higher level of stress-induced protein like DCN-2. Importance of neutrophil (most abundant leukocyte) as an imflammatory marker (neutrophil-lymphocyte ratio) is recognized in previous a study (Guthrie et al., 2013), which is in line with agreement of our present studies. Neutrophil

mediated inflammatory responses *in vivo* have been reported to be regulated by the autophagic phenomenon and degranulation of the neutrophil (Bhattacharya et al., 2015). Not only as noticed in the elevation of TNF-α and IL-6 in the current investigation, other cytokine like interleukin- 1β (IL-1β) also contribute the pathogenesis of atherosclerosis. Cholesterol which plays an important role in atherogenesis also activates neutrophil in crystal form by upregulating the synthesis of this cytokine (Warnatsch et al., 2015). With regard to our current study, it may be suggested that important inflammatory marker like TNF-α production may take place in diversified ways.

The onset of inflammatory response is associated with neutrophil recruitment into infected or injured tissues. Adhesion of circulating neutrophils to the endothelium represents a crucial step in their recruitment into the inflamed tissues. The hypothesis that the upregulation of the anti-adhesive proteins may represent an anti-inflammatory mechanism that contributes to the resolution of inflammation (Pliyev, 2013). In the current study estrogen strongly performed as an anti-inflammatory role. A probable mechanism of the estrogen induced anti-adhesive protein expression via NO mediated pathway may occur in the system.

The results showed that injection of estrogen hormone reduced the TNF-α (Pishva et al., 2016). Lack of estrogen is a cause of cardiovascular disease in men and postmenopausal women. One recent report suggests that estrogen receptor (ER) activation counteracts endothelial dysfunction induced by TNF-α (Palmieri et al., 2014) pointing out the positive outcome of estrogen as noticed in our study. In conclusion, our present result suggests the inhibitory role of DCN-2 on the estriol induction of NO. But when the DCN-2 level is low estriol dominate in the maintenance of higher NO level. The anti-inflammatory effects of

estriol are highly distinct even at a high level of DCN-2 in case TNF-α. Very high level of DCN-2 may nullify the effects of anti-inflammatory effects of estriol, at least in neutrophil. But up to the 100-110 nM of DCN-2, estriol very efficiently supports the NO production and

anti inflammatory responses. Oxidative stress is one of the main applicators for induction of human DCN. In normal physiological conditions a certain level of ROS is regularly produced in the system during normal physiological function or as a metabolic by-product during the cellular respiration mainly by the mitochondrial processes. Hydrogen peroxides and free radicals like superoxide anion etc. have some functions during normal physiological processes as well as in pathological/ infection conditions. Transition metals (i.e. Fe and Cu) which are abundant in the biological system are the potent reactant for H2O2 and free radicals to generate radical-cascade reactions resulting in a number of byproducts (Acharyya et al., 2014). Report reveals that increased extracellular glucose (30 mmol/L) can rapidly stimulate the generation of intracellular ROS through NADPH oxidase and mitochondrial pathways (Susztak et al., 2006). High glucose exposure and cytokine-treatment enhanced the generation of ROS and activation of inflammatory and apoptotic responses in endothelial cells (Busik et al., 2008). ROS in turn can generate the pathological conditions associated with diverse human inflammatory diseases. Stress-induced proteins like antimicrobial peptides/dermcidin have a role to sense the status of the redox balance (Oyinloye et al., 2015).

Taken together, the above results suggested that the effect of stress induced protein dermcidin and the chronic inflammatory products TNF-α, metabolic inflammatory molecules C-reactive protein and oxidative stress inducer malondialdehyde are involved in the

pathogenesis of both T1DM and T2DM. This state ultimately led to the development of acute ischemic heart disease (AIHD), which in turn could be overcome by systemic increase of insulin as reported to be a multifaceted antithrombotic humoral factor (Chakraborty et al., 2004).

Abnormal nutrient metabolism is one of the consequences of diabetes. Atherosclerosis was reported to be the major cause of increased occurrence of cardiovascular and cerebrovascular disorders. Again, hypertension and diabetes mellitus both type-1 and type-2 are reported to be the major risk factors for the genesis of atherosclerosis (Sowers et al., 2001), The development of T1DM due to both increased DCN-2 level and DCN-2 induced inhibition of NO and insulin synthesis might promote prothrombotic condition that leads to atherosclerosis. In this context, it should be mentioned that DCN-2 was found to aggregate platelet at nM concentration through the inhibition of both NO and insulin synthesis (Ghosh et al., 2011). As the level of DCN-2 was very high in T1DM individuals (Fig-7).

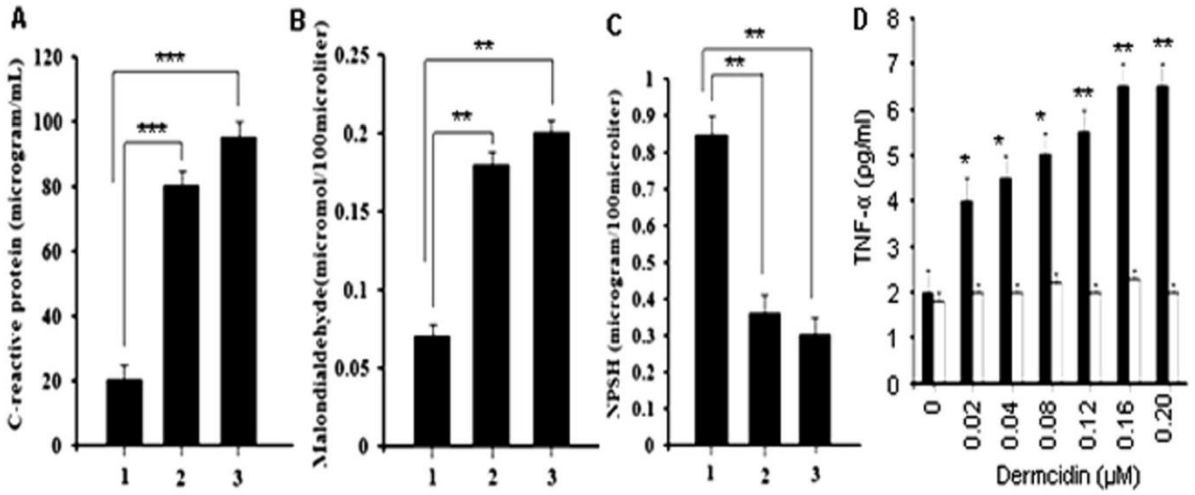

Fig-15. Plasma level of C-reactive protein, malondialdehyde and non-protein soluble thiol in T1DM and normal subjects and DCN-2 induced synthesis of TNF-α in liver cells: The amount of C-reactive protein, malondialdehyde and non-protein soluble thiol present in the CFP of T1DM and normal patients was determined as described in the Materials and Methods. Panel-A: Solid bar '1' represents the amount of C-reactive protein present in the normal volunteers. '2' and '3' represents the amount of C-reactive protein present in the cell free plasma of T1DM volunteers Panel-B: Solid bar '1' represents the amount of MDA present in the normal volunteers. '2' and '3' represents the amount of MDA present in the cell free plasma of T1DM subjects. Panel-C: Solid bar '1' represents the amount of NPSH present in the CFP of normal volunteers. '2' and '3' represents the amount of NPSH present in the cell free plasma of T1DM subjects Panel-D: Solid bars (■) = DCN-2 induced synthesis of TNF-α in liver cell homogenate, hollow bars (□) = 0.9% NaCl induced synthesis of TNF-α in liver cell homogenate. (*Represents $p < 0.05$, ** represents $p < 0.001$,

A recent report suggested that an effective innate immune response can inflate obesity-induced inflammation and various metabolic disorders including diabetes (Kong et

al., 2013). Inflammatory markers such as TNF-α, IL-6 and adipocyte metabolism play an essential role in changes in glucose homeostasis in T2DM individuals (Mohamed et al., 2016). Recent studies revealed that TNF-α may be a very important molecule that is produced by fat cells in obesity and interferes with insulin action (Spiegelman & Hotamisligil 1993; Hotamisligil & Spiegelman, 1994). Infusion of TNF-α to normal rat led to the development of severe hepatic and peripheral insulin resistance (Lang et al., 1992). We have found that most of the diabetic patients show higher level of TNF-α in their plasma and incubation of liver cells with 0.2 μM DCN-2 shows significant elevation of TNF-α synthesis through the up-regulation of TNF-α mRNA by DCN-2 (Fig. 15D). In this respect, it should be mentioned that TNF-α inhibits insulin stimulated glucose transport via the down-regulation of Glut-4 protein (Hotamisligil et al., 1994). This is also supported by the results in the present studies (Fig. 15D). That may result in reduced secretion of insulin from liver and ultimately develop IR and T2DM. Balance between hepatic glucose uptake (HGU) and hepatic glucose production (HGP) has an important role in the regulation of glucose homeostasis, in particular in the post absorptive state. We have found that severity of hyperglycemia is associated with increased high-sensitive C-reactive protein (hs-CRP) in the cell free plasma of diabetic individuals (Fig. 15A). C-reactive protein is regarded as a metabolic inflammatory marker. Taking into the account the note on the TNF-α and IL-6 increase, it may be hypothesized the stress-induced metabolic dysregulations might initiate inflammatory responses. Moreover these two have some interactive relation in the fastening the diabetic pathogenesis. This result suggest that a significant association between metabolic syndrome component with hs-CRP. It was also reported that depletion of serum total bilirubin (TB) is

associated with the enhancement of inflammatory responses and oxidative stresses in T2DM individuals which put severe strain on pancreatic β-cells. The reason of pancreatic β-cells become less responsive is due to the down-regulation of glucose-sensing mechanism in the β-cells (glucotoxicity) leading to diminished insulin secretion. Lower TB may also be the result of impaired liver function (Zheng et al., 2016). A certain degree of impaired liver function may develop insulin resistance. We have also found that most of the diabetic patients showed lower level of plasma non-protein soluble thiol (NPSH) with higher level of oxidative stress marker malondialdehyde (MDA) (Fig. 15B,C) and lipid components (unpublished). Higher level of MDA indicates lipid peroxidation in the subjects with both T1DM and T2DM (Ye, G. et al., 2004). High MDA level is responsible for chronic hyperglycemia either due to lower insulin secretion or due to insulin resistance through the production of excess free radicals (Lim et al., 1998). Our results also demonstrated a positive correlation between high plasma MDA and CRP level with high DCN-2 level in T1DM victims. These suggest that abnormalities in glucose metabolism may impair lipid metabolism, thereby linking obesity to non-insulin dependent diabetes mellitus (NIDDM) or T2DM. But the mechanism that obesity leads to insulin resistance and develop NIDDM or T2DM remains speculative.

Thyroid hormone is known as hyper metabolic hormone which induced high basal metabolic rate and high calorie consumption. This is frequently observing higher BMI or over weigh obese person. In our study high cholesterol and high glucose can induce T3 very high level. The same thing is also notice in Trop-t+ group. So this risk factors group, like high cholesterol and high glucose may result higher CAD or CVD via the T3 mediated hyper metabolic condition. We did not notice any significance change in TSH level or T4 level.

Thyroid hormones play a key role in the hepatic lipid metabolism by a variety of mechanisms. Conversely, hepatic fat accumulation is regarded as the driving force of MS and T2DM-associated dyslipidemia (Taskinen, M.R, 2003; Adiels et al., 2008). These metabolic abnormalities may result in elevated plasma activity of phospholipid transfer protein, an emerging cardiometabolic risk factor which is intricately involved in HDL remodeling, triglyceride metabolism and anti-oxidant status (Dullaart et al., 2008; Vergeer et al., 2010; Dullaart et al., 2013). Although increased fatty acid β-oxidation is anticipated to attenuate hepatic fat accumulation, this process may at the same time result in excessive mitochondrial production of reactive oxygen species. Among other mechanisms, thyroid hormones are likely to affect hepatic lipid accumulation and the subsequent development of fibrosis via an effect on the regulation of adiponectin which stimulates fatty acid oxidation and inhibits de novo lipogenesis (Turer et al., 2012; Mourouzis et al., 2013). Thyroid hormones induce the expression of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, a key regulator of cholesterol synthesis, which is able to convert HMG-CoA to mevalonate (Ness et al., 1973).

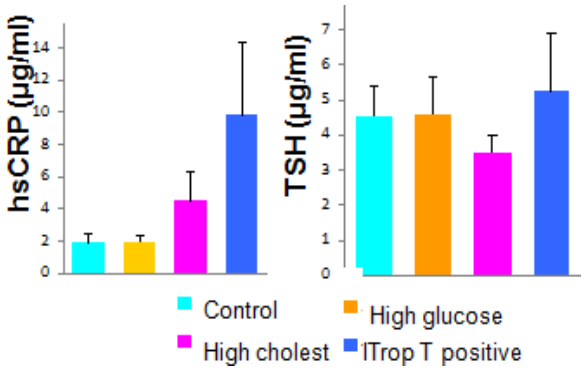

Fig-16. Bar diagram shows the blood serum hs CRP & TSH level comparison among patients with cardiac risk factors & cardiac marker with corresponding the control group. The values are denoted as mean ± SE.

Low density lipoprotein (LDL) receptor expression is also stimulated by thyroid hormones; consequently LDL clearance is increased by the action of thyroid hormones. This results in lower plasma LDL cholesterol in hyperthyroidism and higher levels in hypothyroidism [Duntas, L.H 2000; Duntas & Wartofsky, 2007; Pearce E.N , 2012).

CRP levels have been considered to reflect the extent of inflammatory reactions in the atherosclerotic vessels. Thus, by virtue of its acute phase behavior, CRP is a marker for severity and progression of atherosclerotic processes in the vessels (Heinrich et al., 1995). Finally, the possibility that CRP is linked to cardiovascular disease. Inflammation plays a major role in atherothrombosis, and measurement of inflammatory markers such as high-sensitivity C-reactive protein (hs-CRP) may provide a novel method for detecting individuals at high risk of plaque rupture. Several large-scale prospective studies demonstrate that hs-CRP is a strong independent predictor of future myocardial infarction and stroke among apparently healthy men and women and that the addition of hs-CRP to standard lipid screening may improve global risk prediction among those with high as well as low cholesterol levels. Obesity is associated directly with increased plasma levels of hs-CRP, an observation consistent with findings that adipocytes secrete interleukin-6, a primary hepatic stimulant for CRP production (Yudkin et al., 1999). Indeed, interleukin-6 levels as well as levels of tumor necrosis factor-a have been found to predict risk of first and recurrent coronary events (Ridker et al., 2000). Thus, attenuation of the inflammatory response may represent a mechanism by which diet and weight loss reduces vascular risk.

Diabetic patients have increased levels of hs-CRP which suggests a role for systemic inflammation in diabetogenesis and the insulin resistance syndrome (Festa et al., 2000).

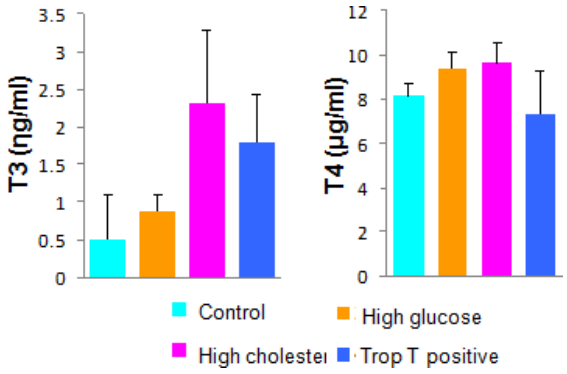

Fig-17. Bar diagram shows the blood serum T3 & T4 level comparison among cardiac risk factors & cardiac marker with corresponding the control group. The values are denoted as mean ± SE.

Finally, growth hormone replacement reduces levels of several inflammatory markers, including hs-CRP, which is of interest because growth hormone–deficient adults have increased cardiovascular mortality (Sesmilo et al., 2000). The metabolic inflammatory marker hs-CRP is increase in high cholesterol and Trop-t+ group suggesting metabolic inflammatory condition may be a disease causing factor.

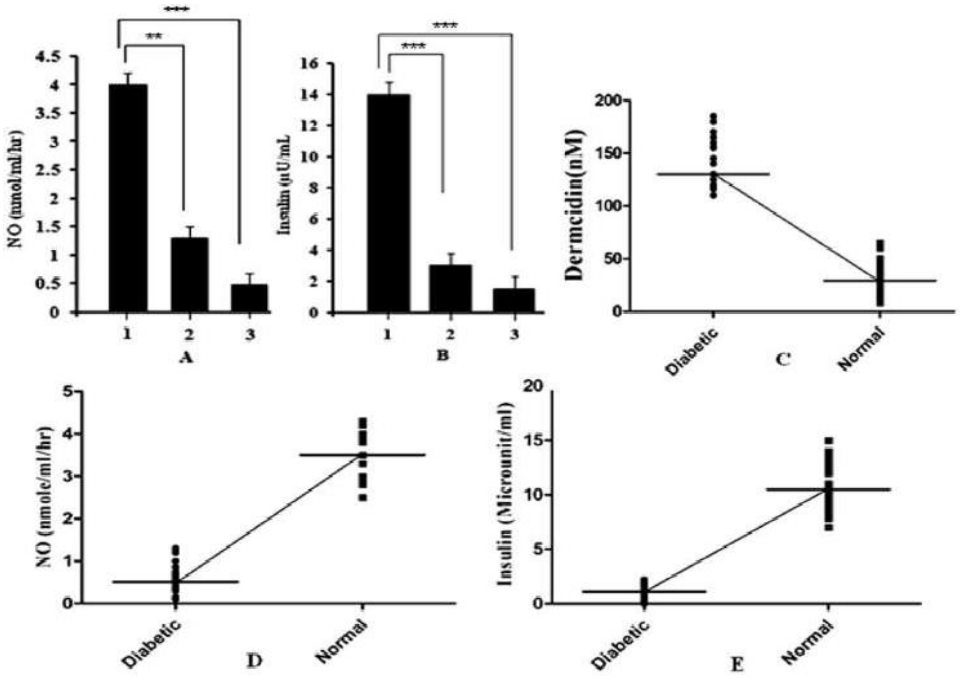

Fig-18. Plasma level of NO and insulin in normal and T1DM volunteers and their correlation with plasma dermcidin level: NO and insulin levels were measured by methemoglobin method and by ELISA in each volunteers as described in Materials and Methods. Panel-A: '1' represents the plasma NO level in normal volunteers. '2' and '3' represents the plasma NO level in T1DM volunteers. Panel-B: '1' represents the plasma insulin level in normal volunteers. '2' and '3' represents the plasma insulin level in T1DM volunteers. Panel-C, D and E represents highly and negatively correlation between plasma dermcidin and plasma NO and insulin level in T1DM and normal subjects. Each point represents the amount of insulin (μU/mL), NO production (nmol/mL/hr) and dermcidin level (nM) of at least 30 age and sex matched normal volunteers with T1DM volunteers (n = 30, male = 15, female = 15). (***Represents $p < 0.0001$, **represents $p < 0.001$).

It is also reported that DCN-2 inhibit insulin synthesis not only in pancreatic β-cells, but also in the hepatic cells of adult mice (Ghosh et al,. 2011). In this context, it should be mentioned here that our result as described in Fig-18 demonstrated a negative correlation between plasma DCN-2 level and plasma insulin and NO level in diabetes patients and a positive correlation between DCN-2 level and blood glucose level in those patients. It is described before that steroid molecule plays an important role in the inhibition of cytokines expression (Robinson et al,. 1993) both through genomic and non-genomic pathway (Jana et al,. 2013). Particularly estrogens, female steroid hormone have a protective role on cardiovascular disease during child bearing ages (Nelson et al,. 2002; Gouva et al,. 2004).

Our present result in Fig-19 suggests the inhibitory role of DCN-2 on the estriol induction of NO. But when the DCN-2 level is low estriol dominate in the maintenance of higher NO level. The anti-inflammatory effects of estriol are highly distinct even at a high level of DCN-2 in case TNF-α. Very high level of DCN-2 may nullify the effects of anti-inflammatory effects of estriol, at least in neutrophil. But up to the 100-110 nM of DCN-2, estriol very efficiently supports the NO production and anti-inflammatory responses.

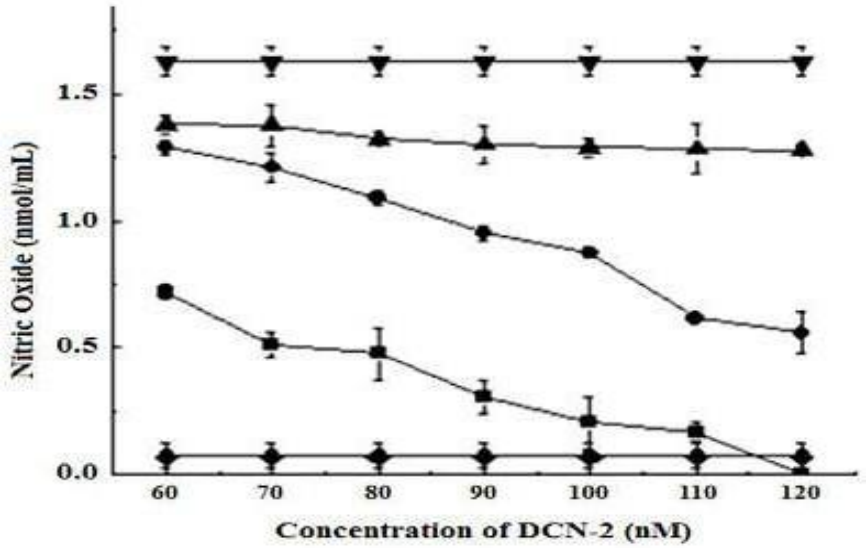

Fig-19. DCN-2 induced inhibition of estriol mediated NO synthesis inneutrophils. Different concentrations of DCN-2 were incubated with neutrophil solution. Solid inverted triangles (-▼-) show synthesis of NO in normal neutrophils with 0.6 nM estriol but in the absence of DCN-2. Solid squares (-■-) describe the change in the synthesis of NO in the absence of estriol but with different concentrations of DCN-2 (80-120 nM) for 120 min. Solid circles (-●-) signify the NO synthesis with different concentrations of DCN-2 followed by 0.6 nM estriol incubation for 45 min. NO synthesis was also carried out with a preincubation of 0.6 nM estriol and different concentrations of DCN-2 for 120 min then which are denoted by solid triangle (-▲-). Solid rhombus (-♦-) describe the synthesis of NO in the control sample were incubated with vehicle only.

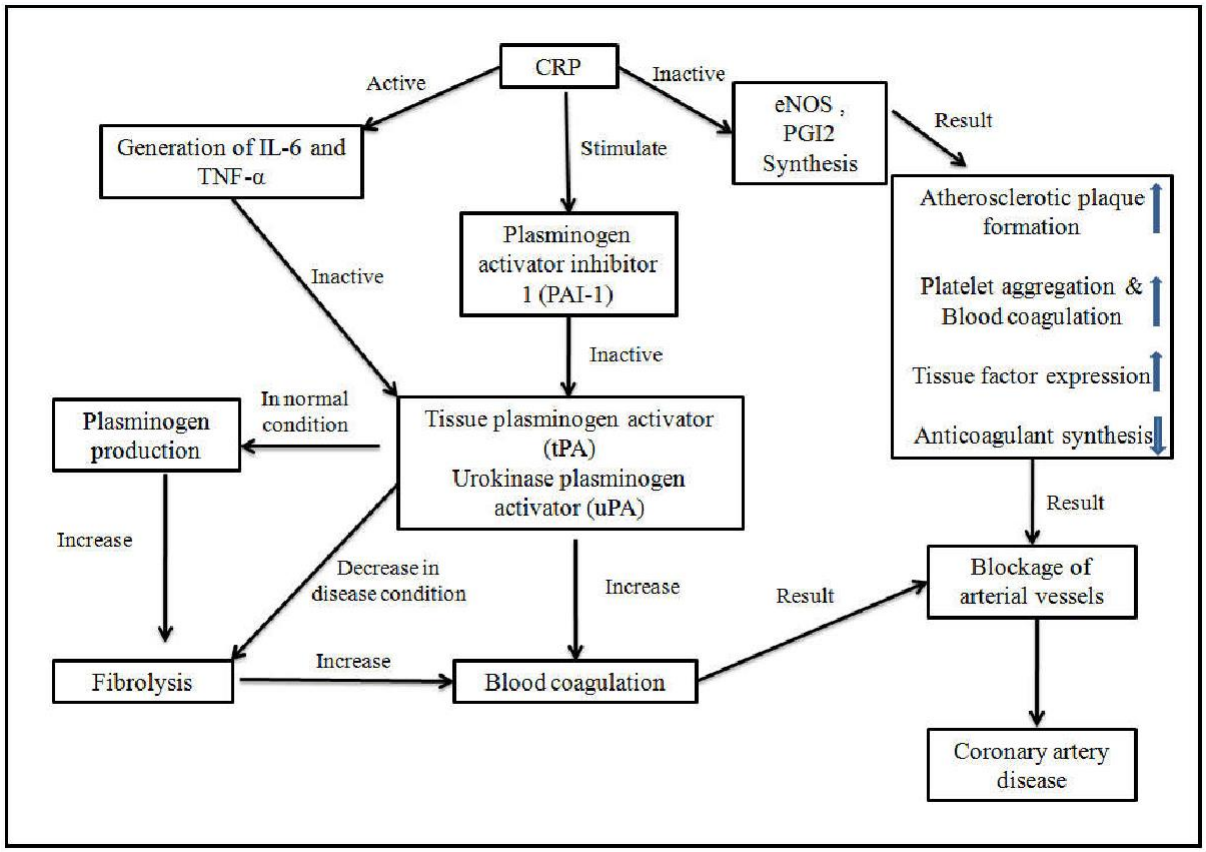

Fig-.20 CRP regulating mechanistic pathway on coronary artery disease via inflammatory and coagulation process.

CRP is the known inflammatory marker and as an independent risk factor for CVD. We focus-out the some possible mechanistic diversified pathway through which CRP act on disease pathogenesis. Several studies demonstrate inflammatory proteins like CRP have been shown to a unique predictor and major cardiovascular pathogenesis like myocardial infarction and death (Ridker et al., 1997; Ridker et al., 2002). So, on the studies of CRP we have special interest to include work.

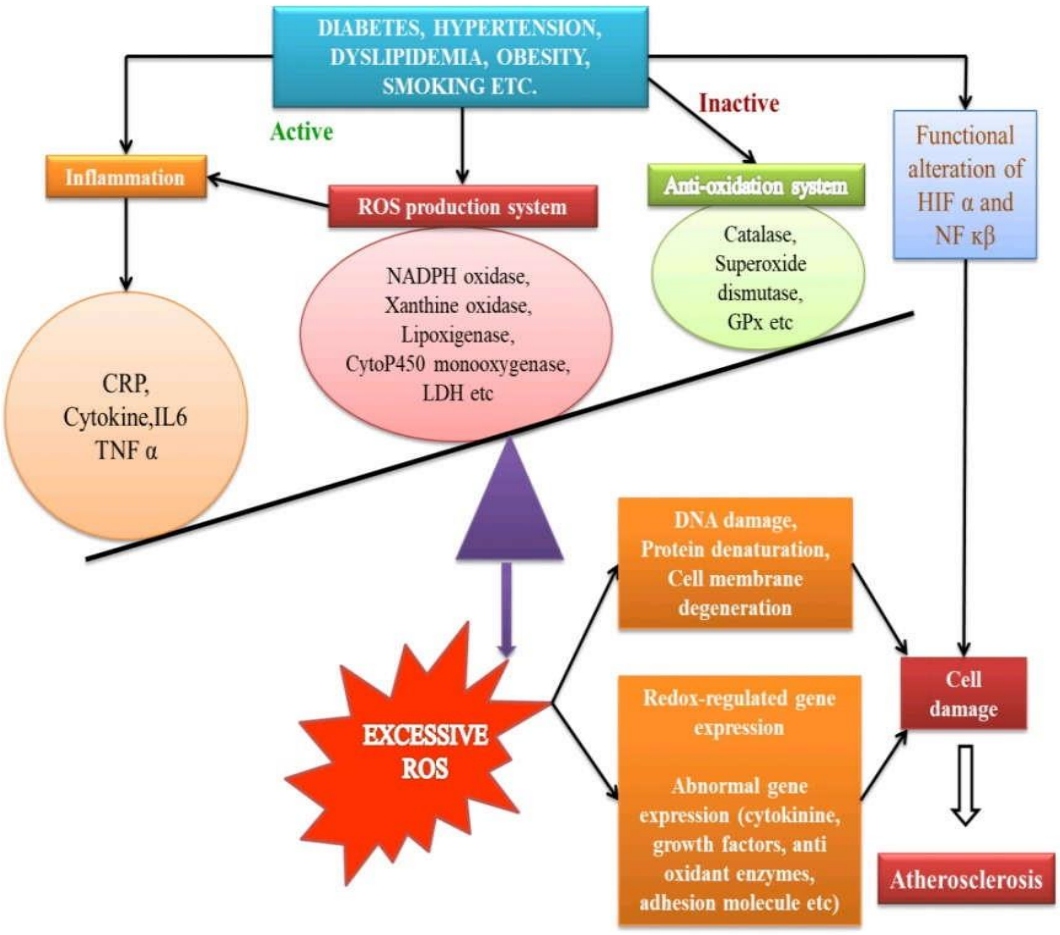

Fig-21. Role of oxidative stress on vascular damage followed by inflammatory responses via excess ROS production.

Fig-21 describes diagrammatically the mechanistic pathways of how diabetes, hypertension, dyslepidemia and smoking may be associated with atherosclerosis via regulation of Inflammatory, redox and antioxidative pathways. Severe diabetes may induce inflammatory responses by influencing CRP, Cytokine, IL-6, TNF-alpha. Excessive of these inflammatory molecules may induce marked increase in ROS generation. As well as disregulation of ROS producing system such as NADPH oxidase, xanthine oxidase, lipoxigenase, Cyt P-450 monooxygenase may contribute to the total ROS pool. Eventually leading to redox regulation of the transcription factors associated with the antioxidants/antioxidant enzyme expression. Engagement of the antioxidant enzymes like SOD/ catalase and molecular antioxidants in the abrogation of oxidative stress causes continuous reduction of them. Finally leading to an imbalanced ROS pool, thus prevailing oxidative stress. This oxidative stress may indulge DNA damages, protein denaturation membrane instability and degeneration leading to cell damage. Large number of genes like growth factor genes, antioxidant genes and adhesion molecules can be influenced either in a positive or a negative way causing an abnormal cell fate leading to atherosclerosis.