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ANTI-ATHEROSCLEROTIC POTENTIAL OF PIOGLITAZONE AND N-ACETYLCYSTEINE

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ABSTRACT

Atherosclerosis involves disturbances in endothelium and the resultant cascade of inflammatory reactions involving generation of ROS (Reactive Oxygen Species). Peroxisome proliferator-activated receptor-γ (PPAR-γ) receptors are more expressed during atherosclerosis process and its stimulation results in inhibition of formation of atherosclerotic plaque. N-acetylcysteine (NAC) stimulates GSH (glutathione) formation and thus helps in scavenging ROS. Thus, the purpose of present study is to explore the role of PPAR-γ agonist (pioglitazone) and N-acetylcysteine in atherosclerosis. Endothelial injury in hyper-lipidemic rat was produced in the femoral artery. The effect of chronic treatment of pioglitazone, N-acetylcysteine and their combination was evaluated by measuring serum HDL, LDL, triglyceride, total cholesterol and lesion index. Pioglitazone and N-acetylcysteine treated rats showed higher levels of HDL and lower levels of LDL, triglycerides and total cholesterol as compared to model control animals (diet + endothelial injury model). Combination of both pioglitazone and N-acetylcysteine produced synergistic action with respect to lipid level. N-acetylcysteine treated animals showed high GSH levels in the liver. Femoral artery lesion index was significantly less in the groups treated with pioglitazone (1.3±0.09), N-acetylcysteine (3.17±0.28) as well as their combination (1.02±0.07) when compared with model control group (4.57±0.19). From all above results, it can be concluded that PPAR-γ agonist (pioglitazone) and N-acetylcysteine are having anti-proliferative, anti-oxidant and/or anti-inflammatory activity which may be responsible for anti-atherosclerotic activity.

INTRODUCTION: Atherosclerosis is a complex multi-factorial process that involves inflammatory and fibro-proliferative responses to various stimuli acting on the vascular wall. Because high plasma concentrations of cholesterol, in particular those of low-density lipoprotein (LDL) cholesterol, are one of the principal risk factors for atherosclerosis, the process of atherogenesis has been considered by many to consist largely of the accumulation of lipids within the artery wall; however, it is much more than that as the lesions of atherosclerosis represent a series of highly specific cellular and molecular responses [1]. Out of the various mediators involved in atherogenesis, PPAR has gained attention in recent future. The Peroxisome proliferator-activated receptors (PPARs) are a subfamily of the 48-member nuclear-receptor superfamily and regulate gene expression in response to ligand binding [2]. Various fatty acids serve as

endogenous ligands for PPARs, whereas some members of the superfamily (farnesoid X receptors) bind bile acids and others (liver X receptors) bind oxysterols. Three PPARs, designated PPAR-α, PPAR-δ and PPAR-γ, have been identified to date. PPAR-α is expressed predominantly in the liver, heart and muscle, as well as in the vascular wall. PPAR-δ is expressed in many tissues, with the highest expression in the skin, brain, and adipose tissue.

PPAR-γ is expressed most abundantly in adipose tissue but is also found in pancreatic beta cells, vascular endothelium and macrophages. Peroxisome proliferator-activated receptor-γ (PPAR-γ), a nuclear receptor, is expressed in all major cell types participating in vascular injury include endothelial cells (ECs), macrophages and vascular smooth muscle cells (VSMCs) [3, 4]. Upon activation, PPAR-γ heterodimerizes with retinoid X receptor, recruits specific co-factors, and binds to responsive DNA elements, thereby stimulating the transcription of target genes.

Activation of this receptor in-vitro inhibits inflammatory processes, including cytokine production and expression of Nitric oxide synthase [5]. In early clinical investigations, ligands of PPAR-γ, such as thiazolidinediones (TZDs), have also been reported to improve endothelium-dependent vasodilatation, suggesting that PPAR-γ activation enhances Nitric oxide (NO) production and protects against vascular injury [6]. Activation of PPAR-γ also inhibits other processes critical for vascular lesion formation include, cell proliferation and migration [7]. PPAR-γ activation by TZDs and 15-deoxy-prostaglandin J_2 inhibits ECs expression of vascular cell adhesion molecule-1, which mediates monocyte adherence to the endothelial surface [8].

Signaling through PPAR-γ, components of oxidized low-density lipoprotein (LDL) increase expression of the scavenger receptor CD36, resulting in lipid accumulation in macrophages [9, 10]. PPAR-γ also activates the macrophage LXR-ABCA1 cholesterol efflux pathway [11] which may explain PPAR-γ ligands inhibit the formation of atherosclerotic lesions in LDL-receptor deficient mice [12]. Coronary artery disease mortality is increased 2 to 4-fold in type 2 diabetes [13]. Atherosclerosis is the major cause of demise in people with diabetes; therefore, it is important to determine the action of any anti-diabetic drug on the atherosclerotic process. Numerous *in-vitro* and *in-vivo* studies support the involvement of LDL oxidation in the pathogenesis of atherosclerosis and also the beneficial effects of antioxidants.

Free-radical mediated lipid peroxidation proceeds by a chain mechanism, where the lipid peroxyl radicals act as chain-carrying species. The mechanism and products of free-radical mediated lipid peroxidation are independent of the initiating species, since the chain propagation is mediated by lipid peroxyl radicals, regardless of whether the oxidation is initiated by attack of hydroxyl radical, peroxynitrite or other radical species. It is therefore important to evaluate antioxidant potency in relation to the type of oxidants and mechanisms [14].

N-acetylcysteine (NAC) is a known free radical scavenger commonly used in paracetamol poisoning and is a component of nutritional supplements. The antioxidant potential of NAC might have beneficial effects in inhibiting atherosclerotic process. Thus, present study is designed to explore/evaluate anti-atherosclerotic potential of PPAR-γ agonist (Pioglitazone) and N-acetyl cysteine using endothelial injury model in rats.

MATERIAL AND METHODS:

Animals: Healthy male Sprague-Dawley untreated rats of weighing 200-250 grams were selected. Three animals per cage were housed in standard 12 hours light and dark cycle with free access standard rat chaw diet (Pranav Agro Industries Ltd., Baroda, India) and water *ad libitum*. The room temperature was maintained at 21 ± 5°C. The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) in accordance to CPCSEA guideline (KBIPER/06/).

Experimental Protocol: Experimental animals were divided into six groups of six animals each as follow:

- **Group I:** (Normal control), Rats were maintained with standard rat chow diet for 30 days.
- **Group II:** (Atherogenic diet), Rats were maintained with high cholesterol diet for 30 days.

- **Group III:** (Model control), Rats were maintained with high cholesterol diet for 30 days and on day 8, endothelial injury was produced in femoral artery.

- **Group IV:** (Pioglitazone treatment group) Rats were maintained with high cholesterol diet for 30 days - on day 8, endothelial injury was produced – Pioglitazone treatment was started on day 9.

- **Group V:** (N-acetylcysteine treatment group) Rats were maintained with high cholesterol diet for 30 days - on day 8, endothelial injury was produced – N-acetylcysteine treatment was started on day 9.

- **Group VI:** (Pioglitazone + N-acetylcysteine treatment group) Rats were maintained with high cholesterol diet for 30 days - on day 8, endothelial injury was produced – combination of Pioglitazone and N-acetylcysteine treatment was started on day 9.

All the animals except for group I were fed high cholesterol diet for 30 days. On the day 8 from initiation of atherogenic diet, endothelial injury was made in the femoral artery of rats in Group III to VI. Groups IV to VI received the test drugs as described above for 21 days. After 21 days of drug treatment, the blood samples were collected for various lipids level measurements and the femoral artery was isolated for visualizing lesion after "Oil Red O" staining under light microscope at 400x magnification.

The composition of standard rat chow diet:

Crude protein (22.9%), Crude oil (4.28%), Crude fiber (3.11%), Ash (7.1%), Sand silica (1.22%). In-house atherogenic diet was prepared as per Paigen *et al*., 1987 [15] with some modification by mixing powdered standard chow diet with cholesterol (1.25%), cholic acid (0.5%) and fat in form of coconut oil (15%). For fat source, coconut oil was selected because of presence of saturated fatty acids include lauric and myristic type, which are responsible for increasing the risk of atherosclerosis.

Procedure for endothelial injury: Endothelial injury in femoral artery was produced as per Merce *et al*., 2000 [16] with some modification. The rats were anaesthetized using mixture of ketamine (70 mg/kg, i.p.) and diazepam (5 mg/kg, i.p.). White hairs on ventral side were removed from lower limb using hair clipper. Femoral artery was identified by pulse using thumb on the medial thigh region. Skin was stretched gently and 1 cm incision was made. Femoral artery was located and separated carefully from the surrounding fascia, femoral vein and nerve using small curved forceps. This process was continued until the vascular bundles were completely separated and artery became free from femoral vein and nerve (**figure 1**). Then, the artery was separated by pair of forceps with blunt tip.

The femoral vein was identified by alternatively applying pressure proximally and distally to distend and collapse the vein. When the femoral artery was isolated, it was clamped with the help of bull-dog clamp. Again twisting of the femoral artery was avoided. Also care was taken that the isolated portion of the artery should remain filled with blood and in between this procedure the isolated artery was continuously kept wet with the help of saline. In the isolated portion of the artery, endothelial injury was made with the help of metal wire, whose surface was made rough and the tip was made blunt and smooth.

Appearance of small drop of blood at the site of incision indicates entry into the lumen of the artery. Take care not to cut the artery excessively or puncture, as only a small hole in the wall of the artery was needed. Metal wire was inserted into the lumen of the femoral artery at a fixed distance and then it was rotated completely for 3 times and removed carefully. After withdrawal of metal wire, back bleeding was allowed through the injury site (to eliminate any potential thrombus or air bubbles).

The femoral artery was ligated proximal to the injury site using 4-0 silk sutures. Then the ligature was tied below the hole in the femoral artery. Thereafter slowly remove the bulldog clamps from the femoral artery. After visual inspection (to ensure adequate pulsation of the femoral artery), the surgical incision was closed with the help of suturing needle and thread, anti-septic cream was applied and the rat was allowed to recover from anesthesia.

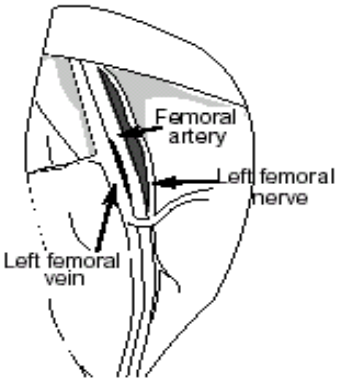

FIGURE 1: SHOWING LEFT FEMORAL ARTERY, VEIN AND NERVE ON LEFT LEG OF ANIMAL

Finally, each rat was placed in the separate cage for a few days and was allowed free to access with provided food and water.

Collection of blood samples: At the end of experimental protocol, animals were kept on 24 hours fasting. Fasting blood samples were collected from retro-orbital sinus in coagulant free eppendorf and allowed to clot at room temperature. The eppendorf tubes were centrifuged at 4000 RPM for 15 minutes at 4ºC to obtain serum. This serum was used to estimate various parameters.

Removal of artery for quantification of Artery Lesion Index: The rats were injected with heparin (5000 IU/kg, i.v.) and were sacrificed by cervical dislocation. The femoral artery of both lower limbs were isolated and immediately immersed in cold Phosphate Buffer Saline (PBS). The arteries were opened longitudinally, pinned out flat, and fixed in 4% PBS (pH 7.2) in formalin for 24 hours at 37°C. After rinsing with water, the fixed femoral arteries were stained with "Oil Red O" for another 24 hours at room temperature, rinsed in 95 % ethanol and were observed under 400X magnification. The stained area was identified as a femoral artery lesion, which had acquired pinkish-orange shade.

Evaluation parameters: Parameters include serum total cholesterol level, triglyceride level, HDL, LDL, reduced glutathione level and artery lesion index (histopathology).

Estimation of Total Cholesterol (mg/dL): Serum total cholesterol level was measured by method described in standard kit (span diagnostic kit). By repeated action of cholesterol esterase and cholesterol oxidase, cholesterol ester of serum gets converted into cholestenone and also produces hydrogen peroxide (H_2O_2). This H_2O_2 then reacts with phenol red and 4-amino antipyrene in presence of enzyme peroxidase which gives red colored quinone.The concentration of cholesterol in serum is directly proportional to the intensity of red colored complex (red quinone) which is measured at 500nm (Jasco-530 spectrophotometer).

Estimation of Serum Triglyceride (mg/dL): Serum triglyceride level was measured by method described in standard kit (span diagnostic kit). Serum TG reacts with LPL (Lipoprotein Lipase) to give glycerol. Glycerol forms dihydroacetone phosphate and H_2O_2 by reacting with glycerol kinase and glycerol 3-phosphate oxidase. This H_2O_2 further reacts with 4-amino antipyrene and peroxidase to give purple colored complex. The intensity of purple color formed during reaction is directly proportional to the TG concentration of serum and is measured at 505 nm (Jasco-530 Spectro photometer).

Estimation of HDL (mg/dL): Serum HDL was measured by method described in standard kit (span diagnostic kit). Low and Very Low Density Lipoproteins (VLDL) were precipitated by a solution containing PEG 6000, leaving behind the High Density Lipoproteins (HDL) in solution. HDL Cholesterol was estimated in the supernatant by a series of enzymatic reactions, which were initiated by the oxidation of cholesterol to cholestenone by cholesterol oxidase, accompanied by the formation of hydrogen peroxide. In a second reaction catalyzed by peroxidase, 4-aminoantipyrene and phenol were reacted with hydrogen peroxide to form red colored quinoneimine. The intensity of red color formed during reaction is directly proportional to HDL concentration of serum and is measured at 505 nm (Jasco-530 spectrophotometer).

Estimation of LDL cholesterol (mg/dL): Serum LDL was measured by method described in standard kit (span diagnostic kit). After determination of serum Total Cholesterol, Triglyceride (TG) and HDL, serum LDL levels in mg/dL was calculated with the help of Fiedman's Formula given in kit leaflet. In which the summation of triglyceride levels after dividing it by 5 and HDL levels were substracted from Total Cholesterol.

Estimation of Reduced Glutathione (µmoles/gm of tissue): The concentration of endogenous reduced glutathione (GSH) level in serum was estimated as per method described by Beutler *et al*., 1963 [17] with some modification. In this method, 2 mL sample was mixed with 10% chilled tri-chloro acetic acid (TCA). The mixture was kept in ice-bath for 30 minutes and centrifuged at 4000 RPM for 10min. at 4˚C. 0.5mL supernatant was mixed with 2 mL of 0.3 M disodium hydrogen phosphate and 0.25 ml DTNB (5, 5'-dithiobis-2-nitrobenzoic acid) (40mg/100ml in 1% sodium citrate) was added and absorbance was measured at 412 nm using spectrophotometrically (Jasco-530 spectrophotometer).

Histopathological Study: The isolated arteries were immediately immersed in cold Phosphate Buffer Saline (PBS). The arteries were opened longitudinally, pinned out flat, and fixed in 4% PBS (pH 7.2) in formalin for 24 hours at 37°C. After rinsing with water, the fixed femoral arteries were stained with "Oil Red O" for another 24 hours at room temperature, rinsed in 95 % ethanol and were observed under 400X magnification. The stained area was identified as a femoral artery lesion, which had acquired pinkish-orange shade.

Hence, the total femoral artery lesion index is defined as the oil red o stained area divided by total area of that femoral artery and multiplied by 100. Processing and staining of the tissue with Oil Red O was carried out according to Paigen *et al*., 1987 [15] with some modification. Similarly lesion area was also quantified by the method of Collin, 2001 [4].

Statistical Analysis: All values were expressed as mean ± SEM. The results were statistically analyzed by unpaired Student's t test. The p values less than 0.05 was considered to be statistically significant.

RESULTS:

Effect of pioglitazone, N-acetylcysteine and their combination on serum triglyceride level: In rats receiving either of pioglitazone (91.91±4.37) or N-acetylcysteine (105.45±5.09) or both (72.92±7.30) the serum triglyceride (TG) levels were significantly lesser than that observed in animals receiving either the atherogenic diet alone or those subjected to endothelial injury (**figure 2**).

Effect of pioglitazone, N-acetylcysteine and their combination on serum total cholesterol level: In rats receiving either of pioglitazone (104.38±5.74) or N-acetylcysteine (128.36±8.02) or both (96.59±5.73) the serum cholesterol levels were significantly lesser than that observed in animals receiving either the atherogenic diet alone or those subjected to endothelial injury (**figure 3**).

Effect of pioglitazone, N-acetylcysteine and their combination on serum HDL level: In rats receiving either of pioglitazone (39.81±1.26) or N-acetylcysteine (31.88±1.68) or both (48.55±3.77) the serum HDL levels were significantly higher than that observed in animals receiving either the atherogenic diet alone or those subjected to endothelial injury (**figure 4**).

Effect of pioglitazone, N-acetylcysteine and their combination on serum LDL level: In rats receiving either of pioglitazone (46.18±3.79) or N-acetylcysteine (75.39±8.68) or both (33.45±8.04) the serum LDL levels were significantly lesser than that observed in animals receiving either the atherogenic diet alone or those subjected to endothelial injury (**figure 5**).

Effect of pioglitazone, N-acetylcysteine and their combination on reduced glutathione level: In rats receiving either N-acetylcysteine (74±6.00) or both (84±6.00) the glutathione levels were significantly higher than that observed in animals receiving either the atherogenic diet alone or those subjected to endothelial injury as well as animals receiving pioglitazone (**figure 6**).

Effect of pioglitazone, N-acetylcysteine and their combination on femoral artery lesion index (histopathology): High amount of lesions were observed in the arterial section obtained from diet +injury group (model control) (4.57±0.19). This character reflecting progression of atherosclerosis was less prominent in groups treated with pioglitazone (1.3±0.09) or N-acetylcysteine (3.17±0.28) or both (1.02±0.07) for 21 days (**figure 7**).

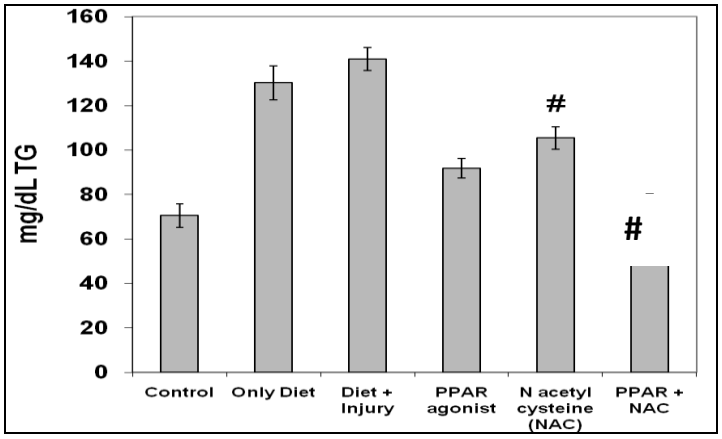

FIGURE 2: EFFECT OF PIOGLITAZONE, N-ACETYLCYSTEINE AND THEIR COMBINATION ON SERUM TRIGLYCERIDE (TG) LEVELS OF FEMORAL ARTERY INJURY MODEL OF RATS COMBINED WITH ATHEROGENIC DIET

Each bar represents the mean ± SEM of six observations. # Significantly different from the results obtained in Diet +Injury (model control) animals at $p < 0.05$.

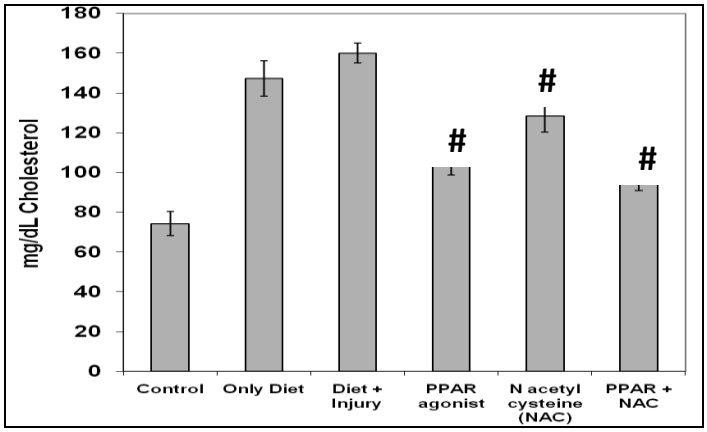

FIGURE 3: EFFECT OF PIOGLITAZONE, N-ACETYLCYSTEINE AND THEIR COMBINATION ON SERUM CHOLESTEROL LEVELS OF FEMORAL ARTERY INJURY MODEL OF RATS COMBINED WITH ATHEROGENIC DIET.

Each bar represents the mean ± SEM of six observations. # Significantly different from the results obtained in Diet +Injury (model control) animals at $p < 0.05$.

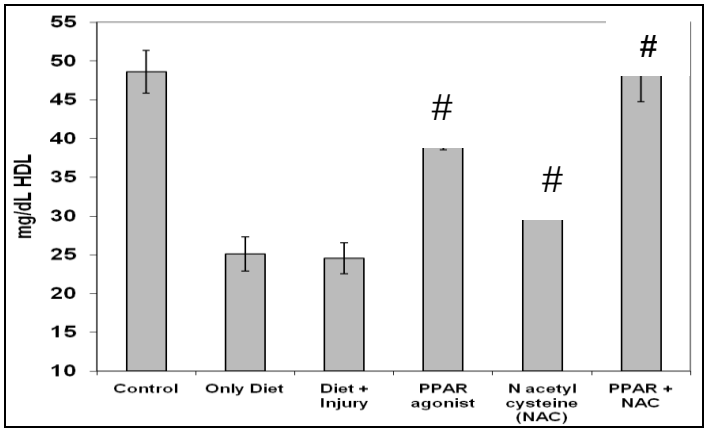

FIGURE 4: EFFECT OF PIOGLITAZONE, N-ACETYLCYSTEINE AND THEIR COMBINATION ON SERUM HDL LEVELS OF FEMORAL ARTERY INJURY MODEL OF RATS COMBINED WITH ATHEROGENIC DIET

Each bar represents the mean ± SEM of six observations. **#** Significantly different from the results obtained in Diet +Injury (model control) animals at $p < 0.05$.

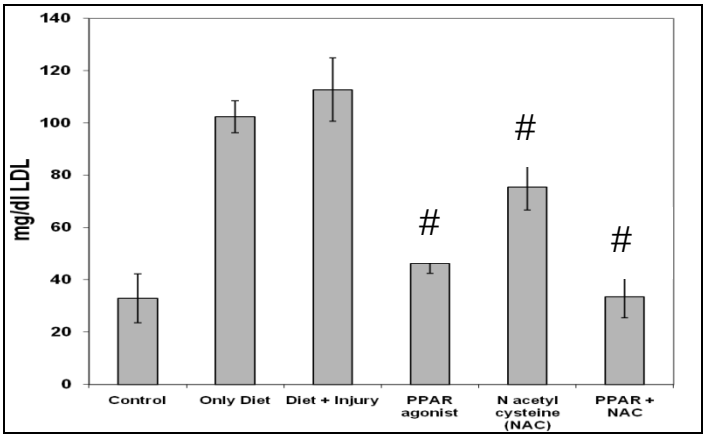

FIGURE 5: EFFECT OF PIOGLITAZONE, N-ACETYLCYSTEINE AND THEIR COMBINATION ON SERUM LDL LEVELS OF FEMORAL ARTERY INJURY MODEL OF RATS COMBINED WITH ATHEROGENIC DIET.

Each bar represents the mean ± SEM of six observations. **#** Significantly different from the results obtained in Diet +Injury (model control) animals at $p < 0.05$.

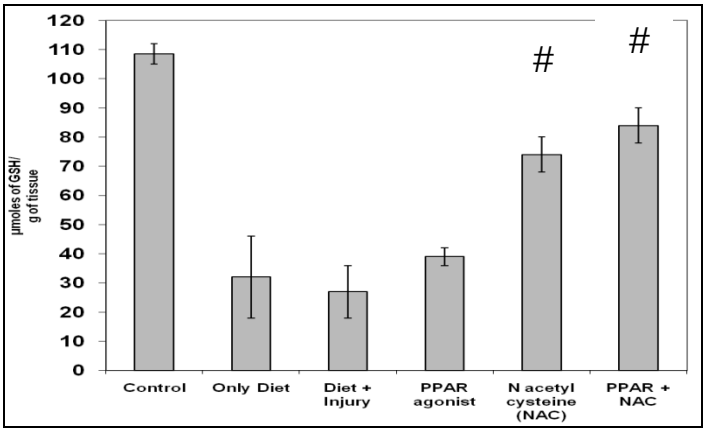

FIGURE 6: EFFECT OF PIOGLITAZONE, N-ACETYLCYSTEINE AND THEIR COMBINATION ON GLUTATHIONE LEVELS OF FEMORAL ARTERY INJURY MODEL OF RATS COMBINED WITH ATHEROGENIC DIET.

Each bar represents the mean ± SEM of six observations. **#** Significantly different from the results obtained in Diet +Injury (model control) animals at $p < 0.05$.

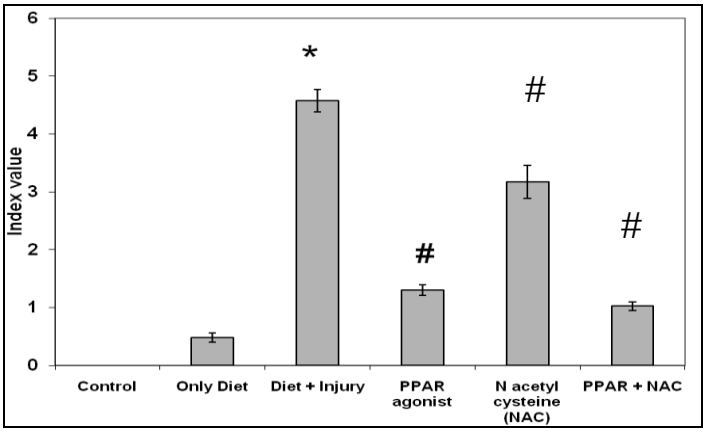

FIGURE 7: EFFECT OF PIOGLITAZONE, N-ACETYLCYSTEINE AND THEIR COMBINATION ON FEMORAL ARTERY LESION INDEX OF FEMORAL ARTERY INJURY MODEL OF RATS COMBINED WITH ATHEROGENIC DIET.

Each bar represents the mean ± SEM of six observations. ***** Significantly different from results obtained in animals given only atherogenic diet at $p < 0.05$. **#** significantly different from the results obtained in Diet +Injury

(model control) animals at $p < 0.05$. In control group, lesions were absent.

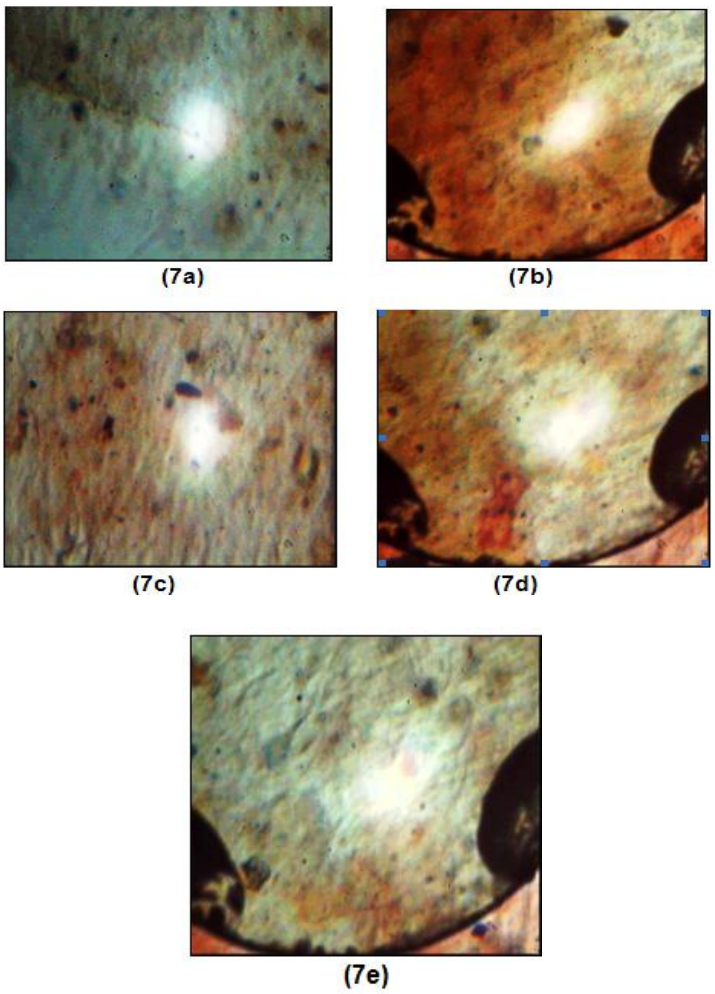

FIG. 7: (7a) ATHEROGENIC DIET GROUP, (7b) ATHEROGENIC DIET + INJURY GROUP, (7c) PIOGLITAZONE TREATED GROUP, (7d) N-ACETYLCYSTEINE TREATED GROUP, (7e) PIOGLITAZONE + N-ACETYLCYSTEINE TREATED GROUP

DISCUSSION: Atherosclerosis is a type of arteriosclerosis in which there occurs accumulation of fatty substance like cholesterol in the inner lining of an artery promoting inflammatory processes and smooth muscle proliferation finally resulting in formation of plaque which may partially or totally block the blood flow through an artery. Higher lipid levels are thus termed as important risk factors in generation of atherosclerotic plaques.

Atherosclerotic plaque formation is thought to be initiated by high lipid levels and endothelial injury. In the present study high cholesterol diet fed to rats raised serum concentration of Cholesterol, LDL and Triglyceride. These when maintained along with femoral artery injury resulted in to atherosclerotic lesions in the artery in a very short span of time [18]. Femoral endothelial injury was produced mechanically with help of specialized metal wire inserted in to the femoral artery.

Blood flow through femoral artery was reinstituted by immediately sealing the punctured site; this type of injury not only causes endothelial dysfunction, but also may distend the artery, similar to a full wall thickness angioplasty injury. Atherosclerotic lesions were visualized by staining femoral artery section with Oil Red O under light microscope. This method of lesion generation and quantification has been well studied by Paigen *et al*., 1987 [15].

Animals who received concurrent treatment with PPAR-γ agonist (Pioglitazone) or N-acetyl L-cysteine or both for 21 days had significantly lower lipid levels as compared to untreated animals, thus both this drugs have significant anti-hyperlipidemic activity. In addition inhibitions of lesion formation in these groups of animals suggest significant anti-atherosclerotic activity.

Food and water intake was normal in all the groups of animals throughout the study period. Increase HDL levels observed with these drugs provide further benefits in inhibiting lesion generation. Out of the two drugs Pioglitazone seems to be more efficacious anti-atherosclerotic drug. NAC though has bit weaker action than Pioglitazone, but when combined with it significantly enhance the anti-atherosclerotic activity of Pioglitazone. Also the combination seems to be better than either of the drugs alone.

As mentioned earlier atherosclerotic lesions involve ROS. ROS promote lesion formation and probably reduced scavenging mechanisms in the body and thus it is responsible for plaque formation. The important ROS endogenous scavenger is reduced glutathione (GSH) whose utilization is significantly increased when ROS formation is increased [19]. Regeneration of GSH from its oxidized state can thus effectively inhibit the oxidation of LDL by ROS.

Glutathione levels were found significantly higher in the animals that received NAC or a combination of NAC and Pioglitazone as compared to untreated animals as well as alone Pioglitazone treated animals. N-acetylcysteine (NAC) is an excellent source of

sulfhydryl (SH) groups, and is converted in the body into SH-metabolites capable of stimulating glutathione (GSH) synthesis, enhance glutathione-S-transferase activity and act directly as free radical scavengers [20, 21].

Our results are in confirmation with the various other reports showing beneficial outcome of thiazolidine-dione treatment in atherosclerosis. PPAR-γ ligands are found to inhibit expression of adhesion molecules required for homing of macrophages in atherosclerotic plaques. Thiazolidinediones like pioglitazone may exhibit anti-inflammatory properties in the vessel wall via the nuclear transcription factor peroxisome proliferator-activated receptor gamma (PPAR-γ).

Better metabolic states generated by these agents along with regulation of the expression of various target genes in vascular cells in vitro and in vivo, subsequently limiting inflammatory cell activation and lesion formation during atherogenesis. Also PPAR-γ ligands are found to inhibit arterial lesions induced by Angiotensin II [7]. The anti-atherosclerotic activity of PPAR-γ ligands could be due to inhibition of production of MMP (Matrix metalloproteinase) [22] and MCP-1 directed macrophage migration [23].

In a recently published study, PPAR-γ agonist treatment significantly lowers the number of lesions in Apo E deficient mice [22]. Additionally in LDL receptor knockout mice receiving high fat diet, treatment with a PPAR-γ ligand (troglitazone) reduced the number of lesions. Similar results were found in nondiabetic mice given a high- fructose diet [4]. Additionally improvement in insulin sensitivity can reduce the risk for plaque formation.

In clinical setup, atherosclerotic patients treated with thiazolidinediones had reduced levels of certain biomarker like CRP (C-reactive protein) suggesting beneficial effects on their patients [24].

CONCLUSION: At last we conclude from our study that pioglitazone and N-acetylcysteine has beneficial effects in inhibition of atherosclerotic plaque formation. The combination of both of these agents seems to be better than N-acetylcysteine alone.

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