Possible Involvement of "Phosphatidylinositol-3 kinase and endothelial nitric oxide synthase" in experimental obesity induced vascular endothelium dysfunction

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ABSTRACT

This study was designed to investigate the possible role of Phosphatidylinositol-3 kinase (PI3K) and endothelial nitric oxide synthase (eNOS) in obesity-induced vascular endothelium dysfunction. Wistar rats were fed high fat diet (HFD) for 12 weeks to induce obesity. HFD induce obesity significantly increased parameters employed viz body weight, basal metabolic index (BMI), total fat pad and lipid profile. Furthermore vascular endothelial dysfunction was assessed in terms of decrease in endothelium dependent relaxation and serum nitrate/nitrite level and increase in mean arterial blood pressure. Atorvastatin (30mg/kg,i.p.) was employed in the present study as standard drug to improve vascular endothelial dysfunction. YS-49 (1.6 mg/kg, i.p.) a specific activator of PI3K and atorvastatin in obese rats significantly improves the lipid profile and markedly improved acetylcholine induced endothelium dependent relaxation, serum nitrite/nitrate concentration and mean arterial blood pressure. However, this ameliorative effect of YS-49 has been prevented by L-NAME (25 mg/kg, i.p.), an inhibitor of eNOS. Further wortmannin (100μg/kg, i.p.),a specific PI3K inhibitor improves the anthropometric parameters and lipid profile but did not show any significant effect on acetylcholine dependent relaxation, serum nitrate/nitrite level and mean arterial blood pressure. Therefore, it may be concluded that PI3K and eNOS pathway is dysregulated in obesity induced vascular endothelial dysfunction and YS-49, a PI3K activator improves vascular endothelial function in eNOS and nitric oxide dependent manner.

Abbreviations

(Ach): Acetylcholine, (Ang-II): Angiotensin II, (BMI): Body Mass Index, (Enos): Endothelial Nitric Oxide, Synthase, (HO-1): Heme Oxygenase, (Kcl): Pottesium Cloride, (MAP): Mean Arterial Pressure, (NO): Nitric Oxide, (PI3K): Phosphatidylinositol 3-Kinase, (SNP): Sodium Nitroprusside, (VED): Vascular Endothelium Dysfunction.

INTRODUCTION

Vascular endothelium dysfunction is characterized by partial or complete imbalance of vasodilator and vasoconstrictor, prothrombotic and antithrombotic factors. Obesity characterized by adipose tissue dysfunction and dyslipidemia is the major causative factor for vascular endothelial dysfunction and cardiovascular morbidity. Adipose tissue dysfunction and dyslipidemia both leads to dysregulation of PI3K pathway. Central or abdominal obesity leads to insulin resistance and endothelial dysfunction through fat-derived metabolic products, hormones, and cytokines.

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Obesity leads to endothelial dysfunction through the frequent association with traditional cardiovascular risk factors and through some more direct novel mechanism (Lassegue et al.,2003). Endothelial dysfunction represents the earliest abnormality in the development of vascular disease, and is pathophysiologically linked to subsequent atherosclerosis progression and cardiovascular disease events (Ferroni et al., 2004). PI3K is an ubiquitous, heterodimeric enzyme that play a pivotal role in the regulation of many cellular processes, including motility, proliferation and carbohydrate metabolism (Abid et al., 2004), Phosphatidylinositol 3-kinase (PI3K) can activate endothelial nitric oxide synthase (eNOS), leading to production of the vasodilator NO. Endothelial phosphatidylinositol 3-kinase (PI3K) can be activated by diverse stimuli such as fluid shear stress (Crackower et al., 2009), estrogen (Hisamoto et al., 2001), & growth factors (Halcox et al., 2001).

The PI3K signaling pathway stimulates the protein kinase Akt, leading to phosphorylation and activation of endothelial nitric oxide synthase (eNOS), resulting in increased production of NO (Murga et al.,2001). Endothelial PI3K can stimulate production of NO via activation of the protein kinase Akt and consequent phosphorylation and activation of eNOS in response to various stimuli (Zeng et al., 2000; Hisamoto et al., 2001).

Therefore, it may be speculated that dysregulation of PI3K may be involved in vascular endothelial dysfunction. YS-49 is a specific PI3K activator that has been reported to have anti-inflammatory, antithrombotic, antiplatelet activity. YS-49 protects cells from oxidant injury; induces heme oxygenase (HO-1) in endothelial cells and protects cells from oxidant grievance via activation PI3K/Akt signaling in vascular cells (Yong et al., 2008). Thus, it is pertinent to investigate the effect YS-49 (PI3K specific activator) alone and with L-NAME (eNOS inhibitor), wortmannin (PI3K specific inhibitor) in obesity induced vascular endothelial dysfunction.

MATERIALS AND METHOD

Experimental Animals

Wistar rats (130-150 g) male, procured from the Central animal house of I.S.F. College of pharmacy, Moga, Punjab, India, were maintained at standard conditions of temperature (25 ± 2 ° C) and humidity (45-55%) and housed at 12/12 hrs light and dark cycle. Animals were acclimatized to laboratory conditions. All the experiments were conducted in approval with Institutional Animal Ethics Committee (IAEC/M1/CPCSEA/P15/2011) and were carried out in accordance with the guidelines of the Indian National Science Academy (INSA) for the use and care of experimental animals.

Experimental Protocol

All animals were divided into different groups each comprising six animals (n=6). (Table: 1) After completion of experimental protocol, anthropometric parameters were estimated. Animals were anaesthetized, blood collected from retro orbital plexuses, centrifuged and serum separated for biochemical estimations. Animals were sacrificed and liver and different fat depots were surgically dissected out and weighed. Thoracic aorta was removed, cut into a ring of 4-5 mm in length and mounted in an organ bath containing krebs- henseleit solution (Tab.1).

Drugs and Chemicals

YS-49, Wortmannin and L-NAME from Sigma Aldrich. Atorvastatin from Ind-swift laboratories Chandigarh, other chemicals and biochemical reagents of analytical grade were used freshly.

High Fat Diet-Induced Obesity

Experimental obesity was induced by feeding high fat diet (containing: powdered normal chow & Lard) (Singh et al., 2011), to rats for a period of 12 weeks.

Assessment of anthropometric parameters

The increase in the body weight, as compared to age matched control group, was regulated as obesity. Body Mass Index (BMI) i.e. weight (g)/height $(cm)^2$ (Novelli et al., 2007) were noted before and after the treatment as an index of obesity. Body weight was assessed weekly. Food intake measurement for individual rat was recorded weekly. To evaluate the effect of high fat diet and drug treatment, adipose tissue (Epididymal, retroperitoneal and mesenteric fat depots) were isolated, freed from surrounding tissues, weighed individually and after that weight was calculated (Ainslie et al., 2000). In addition the liver was isolated, freed from surrounding tissues and weighed.
Body weight: Change in body weight (Weekly) was expressed as % body weight and calculated using following formula:

$$\% \text{ Body weight} = \frac{\text{final b. wt.} - \text{initial b. wt.}}{\text{initial b. wt.}} \times 100$$

Fat pad and liver wt.: Epididymal, Mesenteric, Retroperitoneal and Liver wt. fo 100 (g) b.wt. was calculated using following formula:

$$\text{Fat pad and Liver wt (g)/100 gm b. wt.} = \frac{\text{Weight in gm}}{\text{Final b. wt.}} \times 100$$

Assessment of serum biochemical estimations
Estimation of serum total cholesterol

The total cholesterol was estimated by cholesterol oxidase peroxidise CHOD-POD method (Allain et al., 1974) using commercially available kit (Coral Clinical System. Goa, India).

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{CHE}} \text{Cholesterol} + \text{Free Fatty acids}$$
$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} \text{Cholest-4ene 3-one} + H_2O_2$$
$$H_2O_2 + \text{Phenol} + \text{4-Aminoantipyrine} \rightarrow \text{Red quinoneimime Complex} + H_2O_2$$
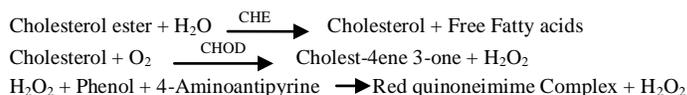
1000 µl of cholesterol reagent was added to 10 µl of serum, 10 µl of standard cholesterol (200 mg/dl) and 10 µl of purified water to prepare test, standard and blank, respectively. All the tubes were incubated at room temperature for 10 mins. The absorbance's of test and standard samples were noted against blank at 505 nm spectophotometrically. Cholesterol is determined after enzymatic hydrolysis and oxidation. Cholesterol esters are hydrolysed by the enzyme cholesterol esterase (CHE) to give free cholesterol and free fatty molecules. This free cholesterol gets oxidized in the presence of cholesterol oxidase (CHOD) to liberate cholest-4ene 3-one and H2O2. The indicator quinoneimime is formed from hydrogen peroxide and 4-Aminoantipyrine in the presence of phenol and peroxides (POD). The intensity of the colour complex is directly proportional to the cholesterol concentration present in sample.

The serum total cholesterol was calculated using the following formula:

$$\text{Total cholesterol (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 100$$

Estimation of serum triglycerides

The serum triglyceride was estimated by glycerophosphate oxidase peroxidise GPO-PAP method (Werner et al., 1981) using commercially available kit (Coral clinical system. Goa, India). 1000 µl of enzyme reagent was added to 10 µl of purified water to prepare test, standard and blank, respectively. all the test tubes were icubated at room temperature for 15 min. The absorbances of test and standard samples were noted against blank at 505 nm spectrophotometrically.

$$\text{Triglycerides} + H_2O \xrightarrow{\text{Lipase}} \text{Glycerol} + \text{Fatty acids.}$$
$$\text{Glycerol} + ATP \xrightarrow{GK} \text{Glycerol-3-Phosphate (G-3-P)} + ADP$$
$$\text{G-3-P} + O_2 \xrightarrow{GPO} H_2O + \text{Dihydroxyacetone phosphate}$$
$$H_2O_2 + \text{Aminoantipyrine} + \text{ESPAS} \xrightarrow{POD} \text{Purplish brown quinoneimime complex} + H_2O + HCL$$
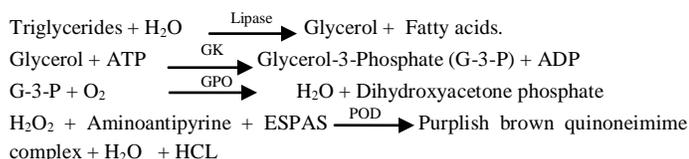
Serum triglycerides are hydrolysed to glycerol and free fatty acids by lipase. In the presence of ATP and glycerol kinase (GK), glycerol is converted to glycerol-3-phosphate which is then oxidised by glycerol-3-phosphate oxidase (GPO) to yield H_2O_2. The H_2O_2, thus formed reacts with 4-aminoantipyrine and N-Ethyl-N-(3-Sulfopropyl)-m-anisidine (ESPAS) in presence of enzyme peroxidise (PDO) to form purplish brown quinoneimime complex, which is measured spectophotometrically.

$$\text{Triglycerides (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 100$$

Estimation of high density lipoprotein (HDL)

The HDL was estimated by cholesterol oxidase peroxidise CHOD-POD method (Allain et al., 1974) using commercially available kit (Coral Clinical system. Goa, India). Step 1; 200 µl of serum and 300µl of precipitating reagent were taken into the centrifuge tube, mixed well and were incubated at room temperature for 5 min and then centrifuged at 3000 rpm for 10 min to get clear supernatant. Step 2; 1000 µl of cholesterol reagent was added to 100 µl of supernatant (from step 1), 100 µl of HDL cholesterol standard (50 mg/dl) and 100 µl of purified water to prepare test, standard and blank, respectively. All the test tubes were incubated at room temperature for 10 min. The absorbance of test and standard samples were noted against blank at 505 nm spectrophotometrically. On addition of the precipitating reagent to the serum, followed by centrifugation, HDL fraction remains in the supernatant while the lipoprotein precipitate out.
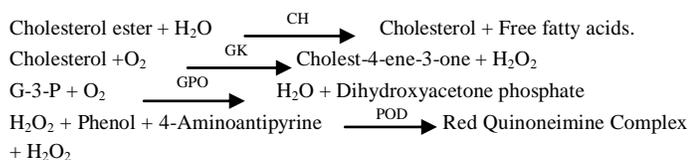
$$\text{Cholesterol ester} + H_2O \xrightarrow{CH} \text{Cholesterol} + \text{Free fatty acids.}$$
$$\text{Cholesterol} + O_2 \xrightarrow{GK} \text{Cholest-4-ene-3-one} + H_2O_2$$
$$\text{G-3-P} + O_2 \xrightarrow{GPO} H_2O + \text{Dihydroxyacetone phosphate}$$
$$H_2O_2 + \text{Phenol} + \text{4-Aminoantipyrine} \xrightarrow{POD} \text{Red Quinoneimine Complex} + H_2O_2$$

Cholesterol is determined after enzymatic hydrolysis and oxidation. Cholesterol esters are hydrolyzed by the enzyme cholesterol esterase (CHE) to give free cholesterol and free fatty molecules. This free cholesterol gets oxidized in the presence of cholesterol oxidase (CHOD) to liberate Cholest-4ene-3-one and H_2O_2. The indicator Quinonenine is formed from hydrogen peroxidise and 4-Aminoantipyrine in the presence of phenol and peroxides (POD). The intensity of the colour complex is directly proportional to the cholesterol concentration present in sample. The serum triglyceride was calculated using the following formula:

$$\text{HDL cholesterol (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 100$$

Assessment of hypertension
Assessment of Mean Arterial Blood Pressure

The rat blood pressure was measured By Tail cuff method (NIBP- BIOPACK MP100, USA). Rat tail was heated with exact time 3 minutes after that tail was inserted in to the cuff same pressure was applied and means arterial blood pressure is recorded on BIOPACK.

Vasoreactivity
Serum Nitrite/Nitrate level using Greiss reagent

Serum nitrite/nitrate was estimated using Greiss reagent which served as an indicator of nitric oxide production.400 µl of carbonate buffer (PH 9.0) was added to 100 µl of serum sample followed by addition of small amount ($\sim$ 0.15 g) of copper-cadmium alloy. The tubes were indubated at room temperature for 1 hour to reduce nitrate to nitrite. The reaction was stopped by adding 100 µl of 0.35 M sodium hydroxide. Following this, 400 µl of zinc sulfate solution (120mM) was added to deproteinate the serum samples, the samples were allowed to stand for 10 min and then centrifuged at 4000 g for 10 min. Greiss reagent (250 µl of 1.0% sulphanilamide and 250 µl of 0.1% napthaylamine diamine dihydrochloric acid in water) was added to aliquots (500 µl) of clear supernatant and serum nitrite/nirate was measured spectrophotometrically at 545 nm. The standard curve of sodium nitrite (10 µM to 100 µM) was plotted to calculate concentration of serum nitrite/ nitrate. Nitrite concentration was calculated using a standard curve for sodium nitrite and expressed as µmol/liter (Sastry et al., 2002).

Effect of Pharmacological Interventions on endothelium dependent and independent relaxation

Isolated rat aortic ring preparation: The rat was decapitated ,thoracic aorta was removed, cut into a ring of 4-5 mm in length and mounted in an organ bath containing krebs-henseleit solution (Nacl,119Mm,Kcl,4.7mM;KH$_2$PO$_4$,1.2 mM and CaCl$_2$,2.5 mM) of PH 7.4,bubbled with carbonated oxygen (95% O$_2$ and 5% CO$_2$) and maintained at 37*C. The preparation was allowed to equilibrate for 90 min under 1.5 g tension (Pieper etal., 1997). The isometric contractions were recorded with a force–transducer (Ft-2040) connected to a physiograph (Inco, Ambala, India) the aortic ring preparation was primed with 80mM Kcl to check its functional integrity and to improve its contractility. The cumulative dose responses of Ach (10^{-8} to 10^{-4} M) or sodium

nitroprusside SNP (10^{-8} to 10^{-4} M) were recorded in phenylephrine (3×10^{-6} M) precontracted (Mitra and Singh, 1998) preparation with intact or denuded endothelium, respectively (Shah and Singh, 2007).

Statistical analysis

All values will be expressed as mean ± S.D. The data for aortic ring preparations were statistically analyzed using repeated measures of ANOVA followed by Newman-Keul's test. The data for aortic glutathione level and serum nitrate/nitrite level, Mean arterial blood pressure (MABP) were statistically analyzed using one way ANOVA followed by Tukey's multiple comparison test. The p value <0.05 was considered to be statistically significant.
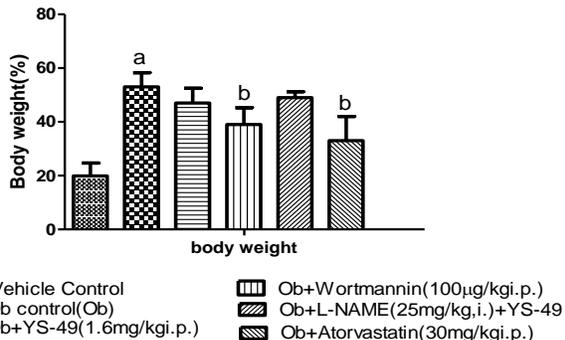
Fig. 1: Effect of pharmacological interventions on body weight. All values are represented as mean ± S.D. a= p< 0.05 vs. Normal control;b= p< 0.05 vs. Ob control.

RESULT

Effect of pharmacological intervention on body weight and Body Mass Index

Administration of HFD (12 weeks) significantly increased body weight and BMI as compared to normal control. YS-49 (1.6mg/kg, i.p.) Wortmannin (100µg/kg, i.p.) and atorvastatin (30mg/kg, i.p.) significantly reduce the body weight and BMI as compared to HFD group. However, L-NAME (25mg/kg, i.p) had no effect on the response to YS-49 on body weight (Fig.-1) and BMI (Fig. 2).

Effect of pharmacological interventions on lipid profile
Assessment of serum triglycerides, serum cholesterol, serum HDL

There was a significant increase (p<0.05) observed in triglyceride and total cholesterol levels and significant decrease in HDL level after 12th week's HFD administration, as compared to rats fed normal chow diet. Treatment with YS-49 (1.6mg/kg, i.p.), Wortmannin (100µg/kg, i.p.) and atorvastatin (30mg/kg, i.p.,) significantly (p<0.05) decreased the serum cholesterol and triglyceride levels and increased the HDL level as compared to HFD control. L-NAME (25mg/kg, i.p.) had no effect on increase in HDL produced by YS-49 (Fig-3, 4, 5).

Changes in weight of fat pad
Twelve week HFD administration produced a significant increase in epididymal, mesenteric, retroperitoneal, and total weight of fat
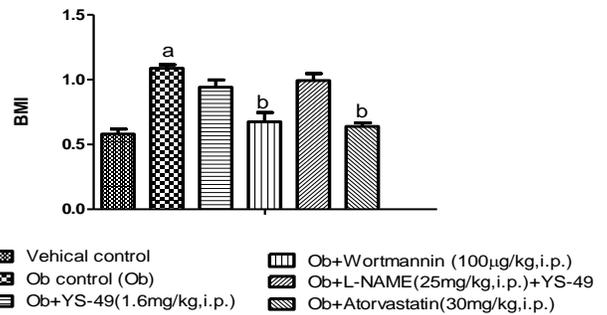
Fig-2: Effect of pharmacological intervention on BMI. All values are represented as mean ± S.D. a= p< 0.05 vs. Normal control;b= p< 0.05 vs. Ob control.
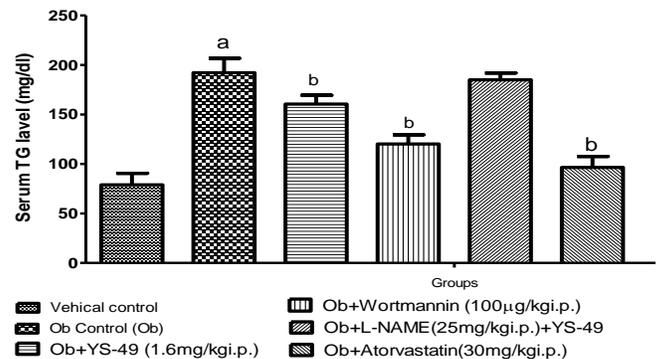
Fig. 3: Effect of interventions on serum triglyceride level (mg/dl). All values are represented as mean ± S.D. a= p< 0.05 vs. Normal control;b= p< 0.05 vs. Ob control.
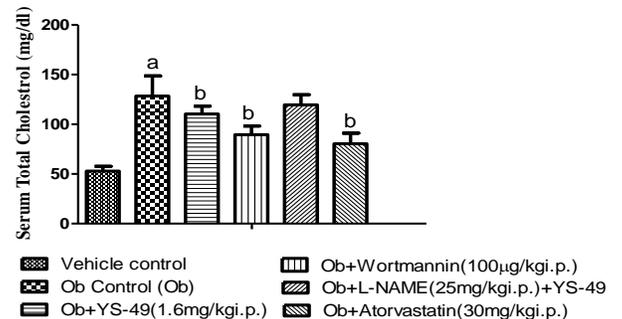
Fig. 4: Effect of pharmacological intervention on serum total cholesterol level (mg/dl). All values are represented as mean ± S.D. a = p< 0.05 vs. Normal control ; b = p< 0.05 vs. Ob control.
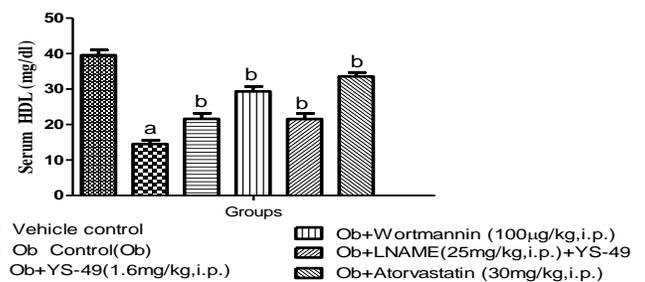
Fig. 5: Effect of pharmacological interventions on serum HDL level (mg/dl). All values are represented as   mean ± S.D. a = p<0.05 vs. Normal control ; b = p< 0.05 vs. Ob control.

pad as compared to vehicle control group. YS-49(1.6mg/kg, i.p.), wortmannin (100µg/kg, i.p.), atorvastatin (30mg/kg, i.p.) significantly attenuated this increase in weight of fat depots as

compare to HFD control group. Effect of YS-49 was completely blocked by L-NAME (25mg/kg, i.p.) (Fig.-6, 7, 8, 9, 10).
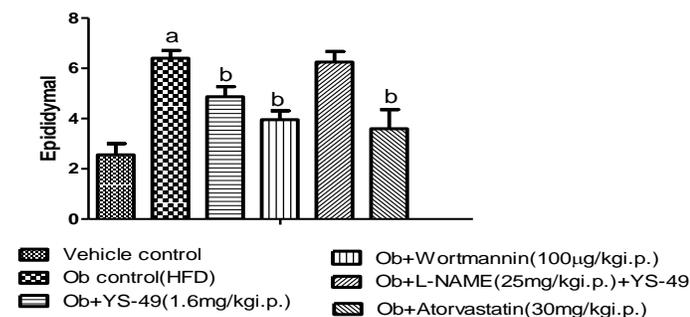
Fig. 6: Effect of pharmacological intervention on epididymal fat. All values are represented as mean ± S.D. a = p< 0.01 vs. Normal control ; b = p< 0.01 vs. Ob control.
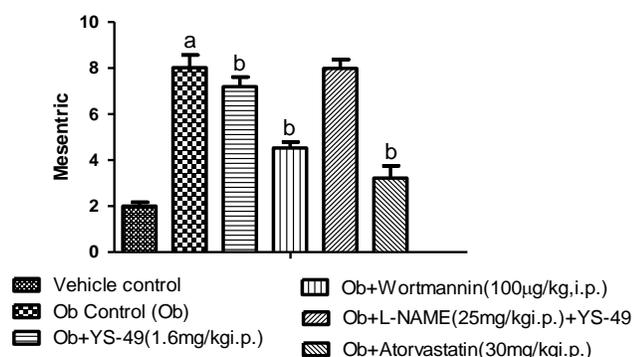
Fig. 7: Effect of pharmacological intervention on mesentric fat. All values are represented as mean±S.D. a=p<0.05vs.Normal control;b=p<0.05 vs.Ob control.
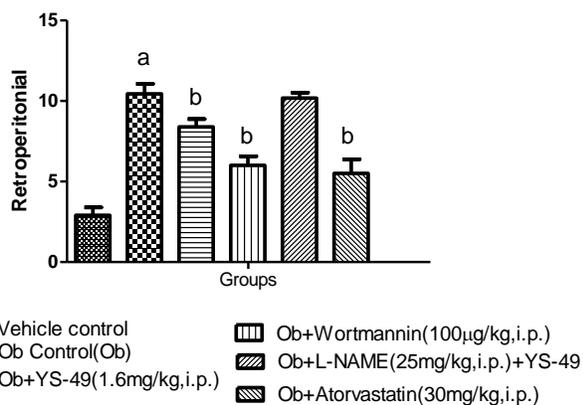
Fig. 8: Effect of pharmacological intervention on retroperitoneal fat. All values are represented as mean ± S.D. a = p< 0.05 vs. Ob control ; b = p< 0.05 vs. Ob control.

Change in liver weight (gm.)/100 gm. body weight

There was a significant increase in liver weight in HFD fed rats. Treatment with YS-49(1.6mg/kg, i.p.) and atorvastatin (30mg/kg, i.p.) significantly attenuated this increase in liver weight. There was no significant effect observed after treatment with wortmannin (100μg/kg, i.p.). L-NAME (25mg/kg, i.p.) significantly attenuated the decrease in liver weight produced by YS-49 (Fig.-10).
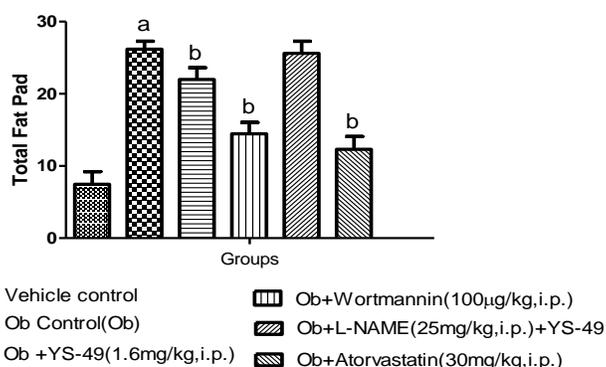
Fig-9: Effect of pharmacological intervention on Total fat pad. All values are represented as mean ± S.D. a = p< 0.05 vs. Normal control ; b = p< 0.05 vs. Ob control.
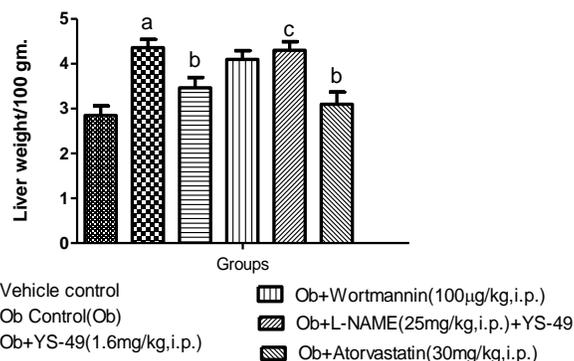
Fig. 10: Effect of pharmacological intervention on Liver weight. All values are represented as mean ± S.D. a = p< 0.05 vs. Normal control; b = p< 0.05 vs. Ob control; c = p< 0.05 vs. YS-49 treated rats.

Mean Arterial Pressure (MAP)

In the present study pharmacological treatment were given to rats for 1 week after 11 week's high fat diet, MAP was significantly increased after HFD administration. YS-49 (1.6mg/kg,i.p.) and atorvastatin (30 mg/kg,i.p.) significantly reduced MAP in comparison to Ob control, PI3K inhibitor, Wortmannin (100μg/kg, i.p.) had no effect on MAP and eNOS inhibitor, L-NAME, (25mg/kg,i.p.) significantly attenuated the decrease in MAP produced the YS-49. (Fig-11).
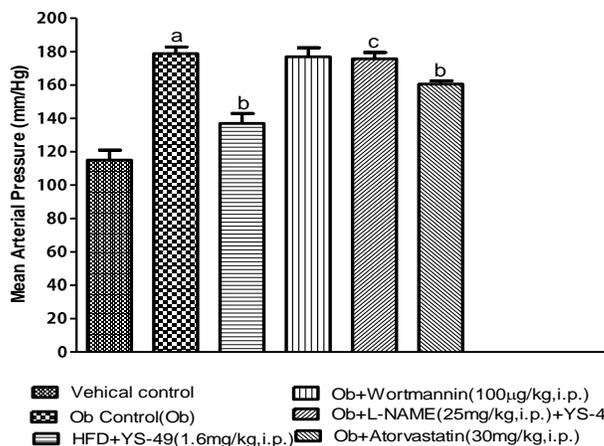
Fig. 11: Effect of pharmacological intervention on Mean arterial pressure. All values are represented as mean ± S.D. a = p< 0.05 vs. Normal control ; b = p< 0.05 vs. Ob control; c = p<0.005 vs. YS- 49 treated rats.

Effect of pharmacological interventions on serum nitrite/nitrate concentration

Experimental obesity significantly reduced serum nitrite/nitrate concentration. Treatment with YS-49 (1.6mg/kg,i.p.) or atorvastatin (30 mg/kg,i.p.) significantly attenuated obesity-induced decrease in serum nitrite/nitrate concentrations.

However L-NAME (25mg/kg,i.p.) significantly prevented the amelioriative effect of YS-49 .Wortmannin (100μg/kg,i.p.) had no significant effect, as compared to Ob control rats (Fig.-12).
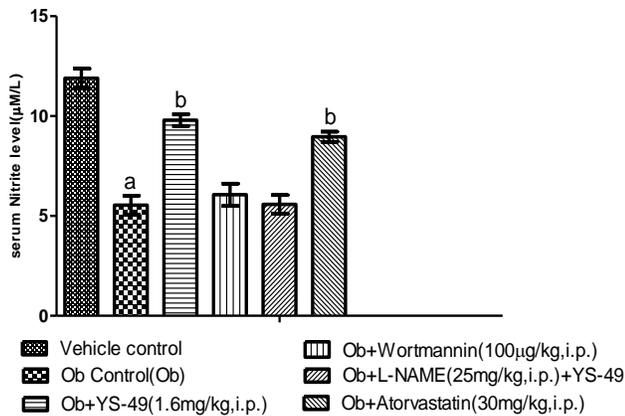
Fig. 12: Effect of pharmacological intervention on Serum nirite/nitrate concentration. All values are represented as mean ± S.D. a = p< 0.05 vs. Normal control ; b = p< 0.05 vs. Ob control.
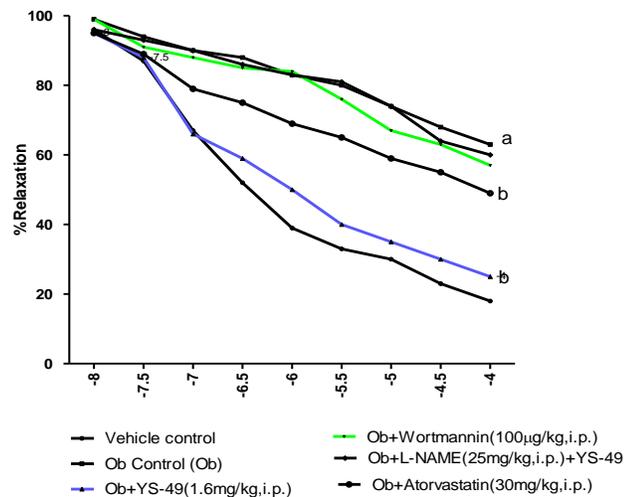
Fig. 13: Effect of Pharmacological Interventions on acetylcholine induced endothelium dependent relaxation in phenyleprine (3×10^{-6} M) precontracted isolated aortic ring preparation. All values (n=6) represent Mean ± S.D. a= p < 0.05 vs.Normal control; b= p < 0.05 vs. Ob control.

Effect of pharmacological interventions on acetylcholine-induced endothelium dependent and sodium nitroprusside-induced endothelium independent relaxation

Acetylcholine (Ach) produced endothelium-dependent relaxation, in a dose-dependent manner, in phenylepherine (3×10^{-6}) precontracted isolated rat aortic ring preparation in normal rats. Experimental obesity significantly attenuated acetylcholine-induced endothelium-dependent relaxation. Administration of YS-

49 (1.6mg/kg,i.p.) and atorvastatin (30 mg/kg,i.p.) significantly attenuated the obesity -induced decrease in acetylcholine-induced endothelium dependent relaxation.

However, L-NAME (25mg/kg,i.p.) significantly prevented the amelorioative effect of YS-49 .Wortmannin , (100μg/kg,i.p.) also reduced acetylcholine-induced endothelium-dependent relaxation but it was not statistically significant. However neither obesity nor any other pharmacological intervention produced any significant change in SNP-evoked endothelium-independent relaxation (Fig.-13, 14)
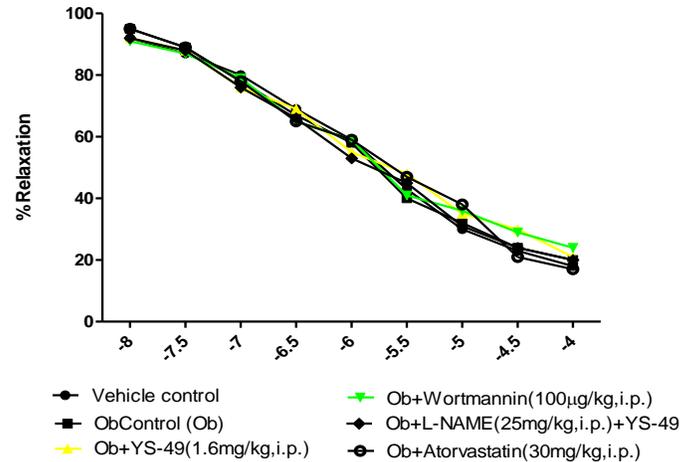
Fig. 14: Effect of Pharmacological Interventions on sodium nitroprusside induced endothelium independent relaxation in phenylephrine precontracted isolated aortic ring preparation. All values (n=6) represent Mean ± S.D.

DISCUSSION

High fat diet induced obesity model is a well reported obesity induction model, and possibly the one that most closely resembles the cafeteria diet obesity in human. The use of high fat diet (HFD) to induce obesity is well documented to cause insulin resistance and hyperglycemia. The first description of HFD to induce obesity by a nutritional intervention was in 1959 (Masek and Fabry., 1959). The Wistar rats were used in the present study because of their small size, low cost and easy availability. Rats fed with a lard-based HFD, develop distinctive visceral adiposity, hyperglycemia, dyslipidemia and obesity (Pang et al., 2008). Thus, in the present study, high fat diet (HFD) for 12 weeks was used to produce obesity and dyslipidemia in wistar rats. Cholic acid stimulates absorption of cholesterol and reduces excretion of cholesterol (Singh et al., 2011). Therefore, high fat diet containing cholic acid was administered to rats to produce Obesity. Obesity is characterized by significant increase in body weight and basal metabolic index (BMI), relative to control animals (Rothwell and Stock, 1982). In the present study both body weight and BMI were significantly increased in HFD fed rats as compared to normal control rats. HFD produces hyperphagia, which may be one important mechanism by which HFD promotes obesity (West and York., 1998). The energy intake is also an important factor in the regulation of body weight. It is well documented that the average energy intake of high fat diet fed rats was higher than that of control fed rats (Milagro et al., 2006).

High fat diet-induced obesity is associated with vascular endothelial dysfunction, as assessed in terms of decrease in acetylcholine induced endothelium-dependent vasodilatation, serum nitrite level and increase in mean arterial blood pressure. The endothelium-dependent vasodilatation has been used as a parameter to assess endothelial function (Singh et al., 2011). Acetylcholine binds to muscranic receptors located on vascular endothelium to produce vasorelaxation. Further, sodium nitroprusside-induced endothelium-independent vasorelaxation has been used as control in this study to investigate the effect of test drugs on endothelium independent vascular reactivity (Sastri et al., 2002). Endogenous formation of nitric oxide is very unstable and gets converted to nitrate and nitrite. Thus serum level of nitrate and nitrite was assessed as an indirect parameter of VED. Dysfunction of vascular endothelial has been used as the index of both dyslipidemia and adipose tissue dysfunction (Ungavari et al., 1999). In obesity the increased number of adipokines, rupture the vascular cells and dysregulate the survival pathway which is associated with the decrease expression of eNOS and NO release (Fleming et al., 2002). Obesity has been documented to produce vascular pathogenesis by interfering with various vascular functions such as decreasing Ach induced vasorelaxation, reduced serum nitrite level/nitrite level and increase iNOS expression and Peroxinitrite levels in vascular smooth muscle cells (Kang et al.,1999) that causes vascular endothelium dysfunction (Shah and Singh, 2007; Sharma et al., 2011). In the present study atorvastatin was used as a standard drug (Furuya et al., 2010). Atorvastatin is known to markedly improve the function of endothelium (Shah and singh, 2007) and reduced total cholesterol level and serum triglyceride level through inhibition of HMG-CoA reductase (Liao et al., 1995). Phosphatidylinositol -3 kinase (PI3K) is a ubiquitous enzyme involved in plethora of cell signaling including the vascular endothelial cells and adipose tissue cells. PI3K regulates phosphorylation and activation of plethora of intracellular signaling targets i.e. proapoptotic factors: BAD, Caspase 9, forkhead transcription factors, NFK-β, mTOR/P70 S6K, eNOS and translocation of Glut-4 (Knight et al., 2006). In recent years, increasing evidence indicates phosphoinositide 3-kinases (PI3K) as crucial signal transducing elements that regulate communication across the plasma membrane. PI3K generate lipid secondary messengers that trigger intracellular responses ranging from metabolic regulation to cell proliferation, survival, and migration. PI3K, plays a major role in endothelial cell and has been reported to activate eNOS (Li et al., 2000). The production of VED by administration of HFD may be due to upregulation in CAPP (PP2A) and down regulation of PKC, Bcl-2, c-jun and PI3K which inactivates eNOS by reducing the generation and bioavailivility of NO and damage the integrity of endothelium. HFD generates ROS by activation of NADPH oxidase (Ignarro et al., 2005). The increased expression of eNOS and hence increased production of nitric oxide, is important for cardiovascular homeostasis, vessel remodeling and angiogenesis (Napoli et al., 2010). Out of various downstream targets of PI3K - endothelial nitric oxide synthatase (eNOS) is specific for endothelial cells.

Thus, it may be speculated that obesity characterized by adipose tissue dysfunction and hypercholesterolemia may disrupt PI3K and eNOS mediated nitric oxide production to cause vascular endothelium dysfunction.The Involvement of eNOS was assessed by using its specific inhibitor L-NAME. As PI3K is also reported to be present in adipose tissue cells, thus the present study included the assessment of the role of PI3K in adipose tissue. The pharmacological treatment with L-NAME (NG-nitro-L-arginine methyl ester) a selective inhibitor of eNOS, has been noted to decrease the effect of YS-49 on vasorelaxation, and the serum nitrate/ nitrite level but it has no effect on body weight, total cholesterol, HDL and triglyceride. Wortmannin a PI3K inhibitor (Sun et al., 2008) was used in the present study to investigate the role of PI3K cell survival pathway, on vascular endothelium dysfunction. Wortmannin reduced the body weight and total weight of fat pad in HFD fed rats. PI3K is a upregulation pathway of obesity in adipose tissue cells. Thus, it is possible that the inhibition of the PI3K by wortmannin, lead to the decrease in obesity, It is well documented that PI3K inhibitor wortmannin blocks the adipocytes differentiation (Asai et al., 2006) and the resultant decreases in adipose mass leads to decreases body weight. In the vasculature, the activation of PI3K increases serine phosphorylation of Akt which, in turn, directly phoshphorylates eNOS on serine 1177 and activates the enzyme, leading to increased NO production (Fulton et al., 1999) YS-49 through PI3K is also known to induce heme oxygenase (HO-1) in endothelial cells and protects cells from oxidant injury. This compound has been reported to inhibit Ang II-stimulated vascular smooth muscle cells (VSMC) proliferation through HO-1 production, which inhibits both the JNK pathway and ROS production. It has been reported that YS-49 confer cardiac protection and anti-inflammatory via activation of the PI3K signal pathway and these effects were antagonized by a PI3K inhibitor, wortmannin (Yan-Choi et al., 2001).

In the present study, YS-49 significantly attenuated the HFD induced increase in lipid profile. This was an unexpected finding, Similar result, were obtained with wortmannin, an PI3K inhibitor. Further work in this area is warranted to delineate the molecular mechanism of this beneficial effect. Further the protective effect of YS49 may also be due to presence of eNOS in endothelial cells YS49 activates PI3K that further activates downstream eNOS to improve vascular endothelium function. Thus, on the basis of above discussion, it is concluded that a decrease in functioning of PI3K and its downstream eNOS pathway plays a significant role in the development of vascular endothelial dysfunction in HFD fed rats. An unexpected new finding was a decrease in lipid profile produced by the PI3K agonist, YS-49.

CONCLUSION

On the basis of the results we can conclude that PI3K is a ubiquitous enzyme and regulate several mechanisms and also has very important role in vasculature. In our study PI3K activator YS-49 improved vascular integrity possibly by optimization of

nitric oxide level and inhibits oxidative stress induced inflammation. An unexpected new finding was the beneficial effect of YS-49, a PI3K agonist, on lipid profile:- a decrease in serum TG and total cholesterol and an increase in serum HDL level. This was almost completely abolished in L-NAME pretreated rats, indicating that it may be due to increased expression of eNOS, produced by YS-49.

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REFERENCES

Abid MR, Guo S, Minami T, Spokes KC, Ueki K, Skurk C, Walsh K, Aird WC. Vascular endothelial growth factor activates PI3K/Akt/forkhead signaling in endothelial cells. Arterioscler Thromb Vasc Biol. 2004; 242: 294-300.

Ainsline DA, Proietto J, Fam BC, Thormburn A.W. Short-term, high-fat diet low circulating leptin concentration in rats. Am.J. Clin. Nutr. 2000; 71: 438-42.

Allain CC, Poon LS,Chan CS, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clin. Chem. 1974; 20: 470-475.

Bernardis LL. Prediction of carcass fat, waterand lean body mass from Lee's nutritive ratio in rats with hypothalamic obesity. Experientia. 1970; 26: 789–90.

Yun-Choi HS, Pyo M K, Park K M, Chang K C, Lee D H. Antithrombotic effects of YS-49 and YS-51--1-naphthylmethyl analogs of higenamine. Thrombosis research. 2001; 4(104), 249 – 255.

Crackower MA, Oudit GY, Kozieradzki I, Sarao R, Sasaki T, Hirsch E, Suzuki A, Shioi T, Irie SJ, Sah R, Cheng H, Rybin O, Lembo G, Fratta L. Regulation of myocardial contractility and cell size by distinct PI3K-PTEN signaling pathways. Cell. 2002; 110: 737-49.

Cypess AM, Lehman S, Williams G, Tal I, Rodman D, Goldfine AB, Kuo FC, Palmer EL, Tseng YH, Doria A, Kolodny GM, Kahn CR. Identification and importance of brown adipose tissue in adult humans. N Engl J Med. 2009; 360(15): 1509-17.

Desjardins F, Balligand JL. Nitric oxide-dependent endothelial function and cardiovascular disease. Acta Clin Belg. 2006; 616: 326-34.

De-wit NJ, Bosch-Vermeulen H, De-Groot PJ, Hooiveld GJ, Bromhaar MM, Jansen J, Muller M, Van-der-Meer R. The role of the small intestine in the development of dietary fat induced obesity and insulin resistance in C57BL/6J mice. BMC Med Genomicd. 2008; 1: 14.

Fulton D, Gratton JP, McCabe TJ, Fontana J, Walsh K, Franke TP, Papapetropolous A, Sessa WC. Regulation of endothelium derived nitric oxide production by the protein kinase Akt. Nature. 1999; 399 (6736): 596-601.

Hall JE, Crook ED, Jones DW, Wofford MR, Dubbert PM. Mechanisms of obesity-associated cardiovascular and renal disease. Am J Med Sci. 2000; 324 (3): 127-3.

Ignarro LJ, Byrns RE, Buga GM, Wood KS, Chaudhuri G. Pharmacological evidence that endothelium-derived relaxing factor is nitric oxide: use of pyrogallol and superoxide dismutase to study endothelium-dependent and nitric oxide-elicited vascular smooth muscle relaxation. J Pharmacol Exp Ther. 1988; 244(1): 181-189.

Kang YJ, Koo EB, Lee YS, Yun-Choi HS, Chang KC. Prevention of the expression of inducible nitric oxide synthase by a novel positive inotropic agent, YS 49, in rat vascular smooth muscle and RAW 264.7 macrophages. British Journal of Pharmacology. 1999; 128: 357 -364.

Kim JH, Hahm D.H, Yang DC, Lee HJ, Shim I. Effect of crude saponins of Korean red ginseng on high fat diet indused obesity in the rat. J Pharmacol Sci. 2005; 97(1): 124-31.

Kizelsztein P, Govorko D, Komarnystsky S, Evans A, Wang Z, Cefalu WT, Raskin I. 20-Hydroxyecdysone decreases weight and hyperglycaemia in a diet induced obesity in mice model. Am J Physiol Endocrinol Metab. 2009; 296: E433-E439.

Knight ZA, Gonzalez B, Feldman ME, Zunder ER, Goldenberg DD, Williams O, Loewith R, Stokoe D, Balla A, Toth B, Balla T, Weiss WA, Williams RL, Shokat KM. A pharmacological map of the PI3-K family defines a role for p110alpha in insulin signaling. Cell. 2006; 125(4): 733-47.

Lassegue B, Clempus RE. Vascular NAD(P)H oxidases: specific features, expression and regulation. Am. J. Physiol. Regulatory integrative Comp. Physiol. 2003; 285: 277-297.

Li JM, Shah AM. Endothelial cell superoxide generation: regulation and relevance and relevance for Cardiovascular pathophysiology. Am.J.Physiol.Regul. Integ.Comp.physiol. 2004; 287: 1014-1030.

Li JM, Shah AM. Endothelial cell superoxide generation: regulation and relevance and relevance for Cardiovascular pathophysiology. Am.J.Physiol.Regul. Integ.Comp.physiol. 2004; 287: 1014-1030.

Liao JK, Zulueta JJ, Yu FS. Peng HB, Cote CG, Hassoun PM. Regulation of bovine endothelial constitutive nitric oxide synthase by oxygen J. Clin Invest. 1995; 96: 2661-2666.

Masek J, Fabry P. High fat diet and the development of obesity in a albino rats. Experientia. 1959; 15: 444-5.

Milagro F I, Campión J, Martínez JA. Weight Gain Induced by High-Fat Feeding Involves Increased Liver Oxidative Stress, Obesity. 2006; 14: 1118–1123.

Novellie LB, Dinij YS, Galhardi CM, Ebaid GMX, Rodrigues HG, Main f, Fernandes AAH, Cicogna AC, Novellifilho JLVB. Anthropometrical parameters and markers of obesity in rats. Lab. Animals. 2007; 41: 111-119.

Pang J, Choi Y, Park T. Ilex paraguariensis exart ameliorate obesity induced by high fat diet: potential rale of AMPK in the visceral adipose tissue. Arch Biochem Biophys. 2008; 476(2): 178-85.

Singh R, Singh AP, Singh M, Krishan P. Impact of obesity on hypertension-induced cardiac remodeling: role of oxidative stress and its modulation by gemfibrozil treatment in rats, Free Radical Biology and Medicine. 2011; 50(2): 363-370.

Rothwell NJ, Stock MJ. Effects of feeding a palatable 'cafeteria' diet on energy balance in young and adult lean (+/?) Zucker rats. Br. J. Nurr. 1982; 41: 461.

Mitra S, Singh M. Possible mechanism of captopril induced endothelium-dependent relaxation in isolated rabbit aorta Mol. Cell. Biochem. 1998; 183: 63–67.

Saiki R, Okazaki M, Iwai S, Kumai T, Kobayashi S, Oguchi K. Effect of pioglitazone on increases in visceral fat accumulation and oxidative stress in spontaneously hypertensive hyperlipidemia rats fed a high fat diet and sucrose solution. J Pharmacol Sci. 2007; 105: 157-67.

Sastry KVH, Moudgal RP, Mohan J, Tyagi JS, Tyagi GS. Spectophotometric determination of serum nitrite and nitrate by copper–cadmium alloy.Anal. Biochem. 2002; 306: 79–82

Sharma S, Singh M, Sharma PL. Ameliorative effect of daidzein: A caveolin-1 inhibitor in vascular endothelium dysfunction

induced by ovariectomy. Indian Journal of Experimental Biology. 2012; 50: 28-34.

        Sowers, JR. Update on the cardiometabolic syndrome. Clin Cornerston. 2011; 4(2): 17-23.

        Sun JJ, Kim HJ, Seo HG, Lee JH, Yun-Choi HS, Chang KC. YS 49, 1-(alpha- naphtyl methyl) -6,7- dihydroxy- 1, 2, 3, 4-tetrahydroisoquinoline, regulates angiotensin II-stimulated ROS production, JNK phosphorylation and vascular smooth muscle cell proliferation via the induction of heme oxygenase-1.Life sciences. 2008; 82: 600-607.

        Werner M, Gabrielson DG, Eastman J. Ultramicro determination of serum triglyceride by bioluminescent assay. Clin. Chem. 1981; 27: 268-271.

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