

## Resveratrol Protects Rabbits Against Cholesterol Diet-Induced Hyperlipidaemia

\* Tanko, Y<sup>1.</sup>, Jimoh, A<sup>1.</sup>, Ahmed, A<sup>2.</sup>, Mohammed, A<sup>1.</sup>, Ayo, J. O<sup>3.</sup>

<sup>1</sup>Department of Human Physiology, Ahmadu Bello University, Zaria, Nigeria, <sup>2</sup>Department of Pharmacognosy and drug development, Ahmadu Bello University, Zaria, Nigeria, <sup>3</sup>Department of Veterinary Physiology and Pharmacology, Ahmadu Bello University, Zaria, Nigeria

**Summary:** The excessive consumption of high cholesterol diet has been associated with an increased incidence of lipidaemia. Lipidaemia is enhanced by formation of oxidative stress, lipid peroxidation and hyperglycaemia. The aim of these experiments was to investigate the protective effect of resveratrol co-administered with cholesterol diet induced hyperlipidaemia in rabbits. Thirty rabbits divided into six groups of five animal (group= 5) each: group 1 = normal control, group 2 = cholesterol diet/high fat diet group only (HFD), group 3 = resveratrol 200 mg/kg (R200), group 4 = resveratrol 400 mg/kg (R400), group 5 = HFD + R200 and group 6 = HFD + R400. The normal group was fed with standard animal feeds only; while the HFD groups were fed with standard animal feeds + cholesterol diet (10% Groundnut oil, 20% Groundnut mill and 2% cholesterol). Resveratrol-treated rabbits received resveratrol suspended in 10 g/L carboxymethylcellulose (CMC) and the control group received the vehicle only, CMC. The preparations were administered for 8 weeks of experimental protocol. At the end of the study period, the animals were sacrificed. Blood and plasma samples were collected. Serum evaluation of lipid profile such as total cholesterol (TC), triacylglycerol (Tg), low density lipoprotein cholesterol (LDL-c) and high density lipoprotein cholesterol (HDL-c) were also assessed. The results obtained show significant ( $P < 0.05$ ) decrease in total cholesterol (TC), Low density lipoprotein cholesterol (LDL-c), total triacylglycerol and an increase in high density lipoprotein cholesterol (HDL-c) in resveratrol treated groups compared to HFD group only. In conclusion, the findings indicated that Resveratrol may contain polar products able to lower plasma lipid concentrations and might be beneficial in treatment of hyperlipidemia and atherosclerosis.

**Keywords:** Cholesterol diet, Lipidaemia, Rabbit; Resveratrol, LDL-c, HDL-c, TC, TG

©Physiological Society of Nigeria

\*Address for correspondence: yusuftanko@abu.edu.ng

Manuscript Accepted: June, 2016

### INTRODUCTION

Regular consumption of food rich in antioxidant are associated with numerous health benefits rooted in their various physiological effects as a result of nutritional constituent (Hunter and Fletcher, 2002). Series of clinical trials have demonstrated the therapeutic benefits of treatment with various antioxidants. Antioxidants showed major improvement in patients with impaired glucose tolerance and lipid metabolisms (Beckman *et al.*, 2003; Liu *et al.*, 2010; Singh *et al.*, 2011). Resveratrol (3, 5, 4'- trihydroxystilbene) is a polyphenol that occurs naturally in foods and drinks made from grapes and peanuts, and also in a number of herbal remedies. The discovery of resveratrol in wine implicated a role for this compound in the 'French Paradox', the observation that the French exhibit a relatively low rate of cardiovascular disease although their diet is high in saturated fats (Siemann and Creasy, 1992). Since then, studies have shown that resveratrol is a member of a class of compound called phytoalexins, which plants use as a defense mechanism against pathogens, and it has also shown that it prevents or

slows the progression of a wide variety of illnesses, including treatment of diabetes complications (Soufi *et al.*, 2012), cancer, cardiovascular disease (Vigtdeux *et al.*, 2010), ischemic injuries and myocardial infarction (Sinha *et al.*, 2002; Lamont *et al.*, 2011), Elevated cholesterol levels have long been recognized as having an association with lipidaemia. Lipidaemia tends to result in an elevation in total cholesterol and triglycerides and a reduction in high-density cholesterol (HDL) (Khaodhiar *et al.*, 2002). Resveratrol has been found to exert a number of potentially cardioprotective effects in vitro, including inhibition of platelet aggregation (Wang *et al.*, 2002), promotion of vasodilatation by enhancing the production of nitric oxide (NO) (Wallerath *et al.*, 2002). Experiments have demonstrated that both melatonin and resveratrol, as found in red wine, protect the heart from myocardial infarction (Lamont *et al.*, 2011). The cardioprotective effect of resveratrol was also attributed to its ability to upregulate the activity of catalase, an antioxidant enzyme in the myocardium (Shigematsu *et al.*, 2003). Increased adipocyte mass is associated with obesity and impaired

lipid metabolism. Consequently, the high levels of circulating free fatty acids (FFA) and glucose are potent inducers of cellular reactive oxygen species (ROS) (Dunmore and Brown, 2013). The aim of this study is to investigate the protective effect of resveratrol co-administered with cholesterol diet induced hyperlipidaemia in rabbits.

## MATERIALS AND METHODS

### MATERIALS AND METHODS

#### Chemicals

All chemicals were obtained commercially and were of analytical grade: Cholesterol (Mumbai India, M. W 386.67, CAS No. 57-88-5, LoT No. 100413) and Mega resveratrol: 99 % pure trans-resveratrol Batch Number: MR 131120, Average particle size: 2.5µm Sigma USA).

#### Materials and instruments

Electronic weighing scale Model: EK 3052 balance, Spectrophotometer, dissecting set, syringes and niddle (Sologuard Medical Device P.V.T Ltd., Chema-600 096, India, ML No. 750).

#### Resveratrol preparation and administration

*Trans*-resveratrol, due to its low solubility in water, was suspended in 10 g/L of carboxymethylcellulose (CMC), and administered orally according to the method of Juan *et al.* (2005).

#### Experimental animals

Seven weeks old male rabbits of different crossbreeds (New Zealand and local breed), weighing between 300 - 350 g, raised in the Animal House, Department of Human Physiology, Ahmadu Bello University, Zaria were used for the study. The animals were kept in well-aerated laboratory cages in the Departmental Animal House, and were allowed to adjust to the laboratory conditions for a period of three weeks before the commencement of the experiment. They were fed with growers' and starters' mash from (Vital Feeds Company Kaduna, Nigeria), and given access to water during the stabilizing period *ad libitum*.

#### Induction of lipidaemia

Lipidaemia was induced by feeding the animals with cholesterol diet for eight weeks. The normal groups were fed with standard animal feeds only, while the high fat-diet groups were fed with standard animal feeds + high fat diet (10 % Groundnut oil, 20 % Groundnut mill and 2 % cholesterol).

#### Experimental Animal Groupings

In the study, 30 rabbits weighing between 300 and 350 g were used, each group comprised five rabbits (n = 5). The animals were grouped according to the method of Joanne *et al.* (2008) as follows:

Group 1: The animals were allowed to free access to a normal diet and received 10 g/L CMC each orally as negative control group

Group 2: The animals were allowed to cholesterol diet/high fat diet (HFD) as feed only and served as positive control group.

Group 3: The animals were allowed to free access to a normal feed and received 200 mg/kg body weight of resveratrol orally (R200).

Group 4: The animals were allowed to free access to a normal feed and received 400 mg/kg body weight of resveratrol orally (R400).

Group 5: The animals were allowed to cholesterol-diet/high fat diet (HFD) as feed only and received 200 mg/kg body weight of resveratrol (R200) orally.

Group 6: The animals were allowed to cholesterol-diet/high fat diet (HFD) as feed only and received 400 mg/kg body weight of resveratrol (R400) orally.

#### Collection and preparation of serum samples for analysis

Eight weeks after the treatment period, all rabbits were subjected to light anaesthesia by exposing them to chloroform soaked in cotton wool placed in anaesthetic box, covered with lid. Blood samples of about 5 ml were drawn from the heart of each sacrificed animal from all groups by cardiac puncture. The samples were collected in Eppendorf tubes and allowed to clot. Thereafter, the serum was separated by centrifugation, using Denley BS400 centrifuge (England) at 3000 g for 10 minutes. The supernatant collected was used for the following analyses:

#### Determination of serum total cholesterol

Total cholesterol (TC) was determined spectrophotometrically, using enzymatic colometric assay kits (Randox Laboratories Limited Kits, United Kingdom). Briefly, the serum level of total cholesterol was quantified after enzymatic hydrolysis and oxidation of the sample according to the method of Stein (1987). Briefly, 1000 µL of the reagent were added to each of the sample and standard. This was incubated for 10 minutes at 20-25 °C after mixing, and the absorbance of the sample ( $A_{\text{sample}}$ ) and standard ( $A_{\text{standard}}$ ) were measured spectrophotometrically against the reagent blank within 30 minutes at 546 nm. The value of total cholesterol present in the serum was expressed in mg/dL. Total cholesterol concentration =  $A_{\text{sample}} / A_{\text{standard}} \times 196.86$  mg/dL.

#### Determination of serum triglyceride

The serum triglyceride level was determined after enzymatic hydrolysis of the sample with lipases as described by Tietz (1990). Briefly 1000 µl of the reagent were added to each sample and standard. This was incubated for 10 minutes at room temperature (20-

25 °C) after mixing, and the absorbance of the sample ( $A_{\text{sample}}$ ) and standard ( $A_{\text{standard}}$ ) were measured against the reagent blank within 30 minutes at 546 nm. The values of triglyceride present in the serum were expressed in mg/dl. Triglyceride concentration =  $A_{\text{sample}}/A_{\text{standard}} \times 194.0$  mg/dL.

**Determination of serum high-density lipoprotein cholesterol**

The serum level of HDL-C was measured using the method of Wacnic and Albers (1978). Low-density lipoproteins (LDL), very low density lipoproteins (VLDL) and chylomicron fractions in the sample were precipitated quantitatively by addition of phosphotungstic acid in the presence of magnesium ions. The mixture was allowed to stand for 10 minutes at room temperature (20-25 °C), and centrifuged for 10 minutes at 1200 g. The supernatant represented the HDL-C fraction. The cholesterol concentration in the HDL fraction, which remained in the supernatant, was determined. The values of HDL-C were expressed mg/dL.

**Determination of serum low-density lipoprotein cholesterol**

The serum level of LDL-C was measured according to the protocol of Friedewald *et al.* (1972) using the equation below:  $LDL-C = TC - (HDL-C + TGL/2.2)$ . The values obtained were expressed in mg/dL.

**STATISTICAL ANALYSIS**

Blood glucose levels were expressed in mg/dL and body weight in kg as mean ± SEM. The data were analyzed using ANOVA followed by Dunett's *post-hoc* test to show multiple comparisons versus control group. Data analysis was evaluated using SPSS version 17.0 software and Microsoft Excel (2007). Values of  $P \leq 0.05$  were considered as significant (Duncan *et al.*, 1977)

**RESULTS**

**Total cholesterol assay**

Figure 1 shows the results of the effects of resveratrol (200 mg/kg and 400 mg/kg) co-administered with high fat diet-fed rabbits. Serum total cholesterol significantly ( $P < 0.05$ ) decreased R200 + HFD and R400 + HFD with values of  $1.56 \pm 0.12$  g/L and  $1.54 \pm 0.14$  g/L when compared to the value recorded for high-fat diet group only with a value of  $2.38 \pm 0.11$  g/L respectively.

**Total triacylglyceride assay**

Figure 2 represents the results of the effects of resveratrol (200 mg/kg and 400 mg/kg) co-administered with high fat diet-fed rabbits. Serum total triacylglycerides showed significant ( $P < 0.05$ ) decrease in values recorded for R200 + HFD and R400 + HFD with values of  $1.28 \pm 0.09$  g/L and  $1.24 \pm 0.07$

g/L when compared to high fat diet group only with a value of  $1.64 \pm 0.81$  g/L.

**High-density lipoprotein cholesterol assay**

Figure 3 shows the results of the effects of 200 mg/kg and 400 mg/kg of resveratrol co-administered with high fat diet-fed rabbits. Serum high density lipoprotein cholesterol showed significant ( $P < 0.05$ ) increase in resveratrol co-administered with values of  $0.54 \pm 0.02$  g/L for HFD + R200 and  $0.52 \pm 0.08$  g/L for HFD + R400 when compared to high fat diet group only with a value of  $0.38 \pm 0.04$  g/L.

**Low density lipoprotein cholesterol assay**

Figure 4 shows the results of the effects of resveratrol (200 mg/kg and 400 mg/kg) co-administered with high fat diet in rabbits. Serum low density lipoprotein cholesterol showed significant ( $P < 0.05$ ) increase in R200 + HFD and R400 + HFD with values of  $0.32 \pm 0.15$  g/L and  $0.41 \pm 0.16$  g/L when compared to high fat diet group only with a value of  $1.25 \pm 0.09$  g/L.

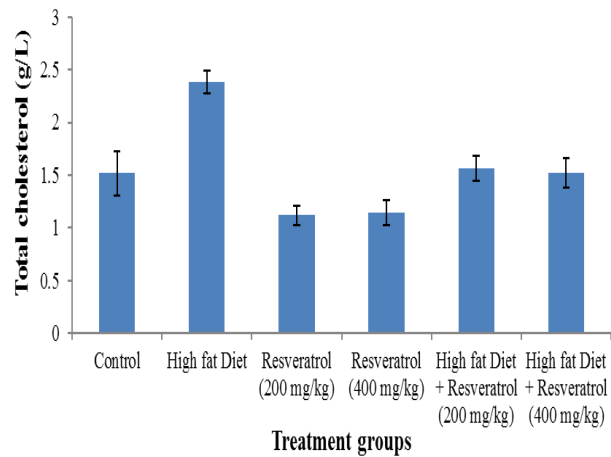


Figure 1: Effect of co-administration of resveratrol and high-fat diet on total cholesterol in rabbits as compared with fed and unfed control group. Values are expressed as mean ± SEM; n = 5 Values with error bars having different superscripts letters are significant <sup>a,b,c</sup> = p < 0.05 significant.

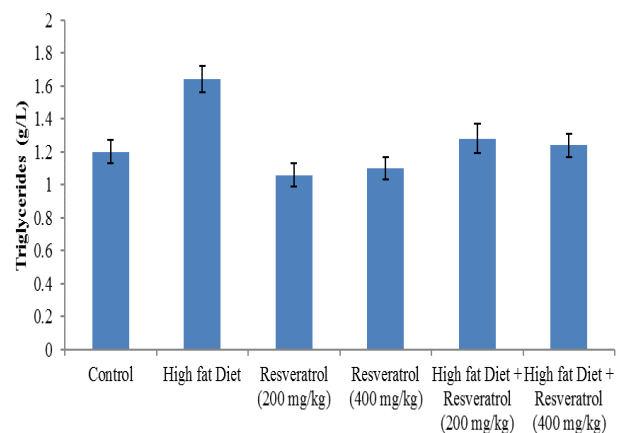


Figure 2: Effect of co-administration of resveratrol and high-fat diet on total triacylglyceride in rabbits as compared with fed and unfed control group. Values are expressed as mean ± SEM; n = 5.

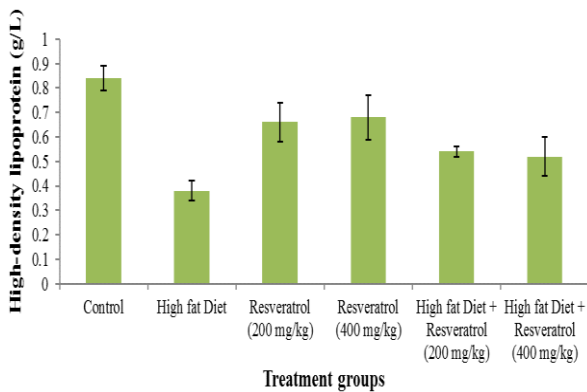


Figure 3: Effect of co-administration of resveratrol and high-fat diet on high density lipoprotein in rabbits as compared with fed and unfed control group. Values are expressed as mean  $\pm$  SEM; n = 5.

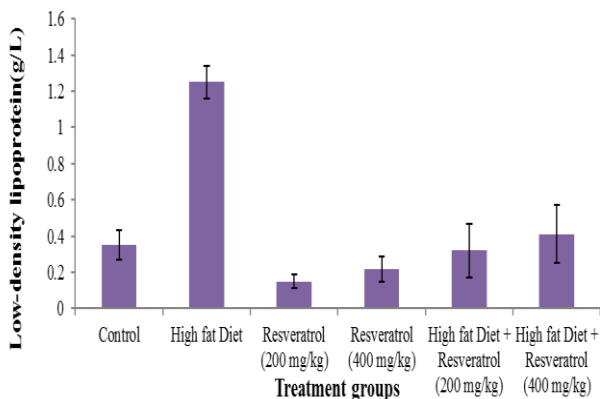


Figure 4: Effect of co-administration of resveratrol and high-fat diet on low density lipoprotein in rabbits as compared with fed and unfed control group. Values are expressed as mean  $\pm$  SEM; n = 5.

## DISCUSSION

The lipid profile obtained in the present study showed a significant decrease in total cholesterol, total triglyceride, low-density lipoprotein and an increase in high-density lipoprotein cholesterol levels in resveratrol groups' treated with cholesterol diet compared to cholesterol diet group only. Lipidaemia observed in cholesterol diet group may be as a result of increase in visceral adipose mass (Brown and Dunmore, 2013), which causes impaired lipid metabolism and consequently result in high levels of circulating free fatty acids and glucose which are potent inducer of cellular reactive oxygen species (Youn *et al.*, 2014). High level of free fatty acid causes elevated cholesterol levels which have long been recognized as having an association with hyperlipidaemia (Buettner *et al.*, 2006). The observed decrease in total cholesterol, total triacylglycerol, low density lipoprotein and increase in high density lipoprotein in groups supplemented with resveratrol may be due to low activity of cholesterol biosynthesis enzymes, low level of lipolysis and inhibition of

Resveratrol prevents hyperlipidaemia in rabbits

dysregulation of lipid metabolism which hindered mobilization of excess cholesterol into the body system. These findings suggest that one of the possible mechanisms of anti-lipidaemic action of resveratrol supplement is by modulating one or more of the aforementioned mechanisms. The decrease in lipid profile after consumption of cholesterol diet with resveratrol may have demonstrated why resveratrol, as found in red wine, protects the heart from myocardial infarction (Lamont *et al.*, 2011). This cardio-protective effect of resveratrol may also be attributed to its ability to upregulate the activity of catalase, an antioxidant enzyme in the myocardium (Shigematsu *et al.*, 2003). Hyperlipidaemia itself usually causes no symptoms, but may lead to symptomatic vascular diseases, including coronary artery disease and peripheral arterial disease (Rohilla *et al.*, 2011). Jeong *et al.* (2011), demonstrated that administration of grape skin extract rich in resveratrol to animal fed on high fat diet significantly recorded a decrease in total cholesterol, triglyceride, low density lipoprotein and increase in high density lipoprotein which agrees with our findings. The decrease in serum lipid profile may be as a result of resveratrol inhibiting fat accumulation and fatty acid synthesis by activation of fatty acid oxidation, demonstrated by Sahar and Abdel, (2012).

In conclusion, the findings indicated that Resveratrol may contain polar products able to lower plasma lipid concentrations and might be beneficial in treatment of hyperlipidemia and atherosclerosis.

## REFERENCES

- Beckman, J. A., Creager, M. A. and Libby, P. (2003). Diabetes and atherosclerosis: epidemiology, pathophysiology and management. *Journal of American Medical Association*, 287: 2570-2581.
- Buettner, R., Parhofer, K. G., Woenckhaus, M., Wrede, C. E., Kunz-Schughart, L. A., Scholmerich, J. and Bollheimer, L. C. (2006). Definition high fat-diet rat models: metabolic and molecular effects of different fat types. *Journal of Molecular Endocrinology*, 36: 485-501
- Duncan, R. C., Knapp, R. G. and Miller, M. C. (1977). Test of hypothesis in population means. In: *Introductory Biostatistics for the health sciences*, John Wiley and Sons Inc. NY: 71-96
- Dunmore, S. J. and Brown, J. E. P. (2013). The roles of adipokines in  $\beta$ -cell failure of type 2 diabetes. *Journal of Endocrinology*, 216: 37-45.
- Friedewald, W. T., Levy, R. and Fredrickson, D. S. (1972). Estimation of concentration of low density lipoprotein. *Fundamentals of Clinical Chemistry*. W. B Saunders Philadelphia, 3: 470-479.
- Jeong, Y. S., Jung, H. K., Cho, K. H., Youn, K. S. and Hong, J. H. (2011). Anti-obesity effect of grape

- skin extract in 3T3-L1 adipocytes. *Food Science Biotechnology*, 20: 635-642.
- Huntler, K. J. and Fletcher, J. M. (2002). The antioxidant activity and composition of fresh, frozen, jarred and canned vegetable. *Innov Food Sci. Emerg. Technol*, 3: 399-406.
- Joanne, M. A., Xiaomei, L., Christopher, Q. R., Brandi, P. and Min, Y. (2008). Resveratrol alleviates alcohol fatty liver in mice. *American Journal of Physiology*, 295(4): 833-842.
- Juan, M. E., Gonzalez-pons, E., Munuera, T., Ballester, J., Ridnguez-Gil, J. E and Planas, J. M. (2005). Trans- resveratrol, a natural antioxidant from grapes, increases sperm output in healthy rats. *Journal of Nutrition*, 135: 757-760.
- Khaodhiar, L., McCowen, K. C. and Blackburn, G. L. (2002). Obesity and its comorbid conditions. *Clinical Cornerstone*, 2: 17-31.
- Lamont, K. T., Somers, S., Lacerda, L., Opic, L. H. and Lecour, S. (2011). Is red wine a safe sip away from cardioprotection? Mechanisms involved in resveratrol-and melatonin-induced cardioprotection. *Journal of Pineal Research*, 50(4): 374-380.
- Liu, F., Hung, L., Wu, W., Chang, D., Huang, C., Lai, J. and Ho, L. (2010). Chondroprotective effects and mechanisms of resveratrol in advanced glycation end products-stimulated chondrocytes. *Arthritis Research Therapy*, 12(5): R167
- Rohilla, A., Rohilla, S., Singh, G., Kumar, A. and Khan, M. U. (2011). Atorvastatin pleiotropism role in cardioprotection. *International Journal of Pharmaceute Biology Achieves*, 2(3): 813-818
- Sahar, S. and Abdel, M. S. (2012). The effects of varieties sources of omega-3 fatty acids on diabetes in rats. *Food and Nutrition Sciences*, 3: 1404-1412.
- Sinha, K., Chaudhary, G. and Gupta, Y. K. (2002). Protective effect of resveratrol against oxidative stress in middle cerebral artery occlusion model of stroke in rats. *Life Science*, 71: 655-665.
- Shigematsu, S., Ishida, S., Hara, M., Takashi, N., Yoshimatsu, H., Sakata, T. and Korthuis, R. J. (2003). Resveratrol, red wine constituent of polyphenol, prevents superoxide-dependent inflammatory responses induced by ischemia/reperfusion, platelet-activating factor or oxidants. *Free Radical Biology and Medicine*, 34: 810-817.
- Singh, G., Gupta, V., Sharma, A. K. and Gupta, N. (2011). Frequently of thyroid dysfunction among diabetes in punjabi population. *Advance in Bioresearch*, 2: 3-9.
- Siemann, E. H. and Creasy, L. L. (1992). Concentration of phytoalexin resveratrol in wine. *American Journal of Enology and Viticulture*, 43: 49-52.
- Soufi, F. G., Mohammed-Nejad., D. and Ahmadiéh, H. (2012). Resveratrol improves diabetic retinopathy possibly through stress-nuclear factor KB-apoptosis pathway. *Pharmacological Report*, 64: 1504-1514.
- Stein, E. A. (1987). Lipids, lipoproteins and Apolipoproteins. In: Treitz, N. W. (Ed). *Fundamentals of Clinical Chemistry*, 3rd Edn., W. B Saunders Philadelphia, pp; 470-479.
- Tietz, N.W. (1990). Clinical Guide to Laboratory Test, Second Edition W.B. Saunders Company, Philadelphia, U.S.A. 554-556.
- Vingtdeux, V., Giliberto, L., Zhao, H., Chandakkar, P., Wu, Q., Simon, J. E., Janle, E.M., Labo, J., Ferruzzi, M. G., Daries, P. and Marambaud, P. (2010). Amp-activated protein kinase signaling activity by resviratrol modulates amyloid-beta peptide metabolism. *Journal of Biological Chemistry*, 285(12): 9100-9101.
- Wacnic, R. G. and Alber, J. J. (1978). A comprehensive evaluation of the heparin manganese precipitation procedure for estimating high density lipoprotein cholestrol. *Journal of Lipid Research*, 19: 65-76.
- Wang, J., Obici, S., Morgan, K., Barzilai, N., Feng, Z. and Rossetti, L. (2002). Overfeeding rapidly induces leptin and insulin resistance. *Diabetes*, 50(12): 2786-2791.
- Wallerath, T., Deckert, G., Ternes, T., Anderson, H., Li, H., Witte, K. and Forstermann, U. (2002). Resveratrol, a polyphenolic phyto alexin present in red wine enhances expression and activity of endothelial nitric oxide synthase. *Circulation*, 106(13): 1652 - 1658.
- Youn, J. Y., Siu, K. L., Lob, H. E., Itani, H., Harrison, D. G. and Cai, H. (2014). Role of vascular oxidative stress in obesity and metabolic syndrome. *Diabetes*, 63: 2344-2355.