Plant Archives Vol. 20, Supplement 1, 2020 pp. 3157-3164 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

ANISOTES TRISULCUS PREVENTS HYPERCHOLESTEROLEMIA, OXIDATIVE STRESS AND INFLAMMATION AND MODULATES FATTY ACID SYNTHASE IN HIGH CHOLESTEROL DIET-FED RATS

Mousa O. Germoush
Biology Department, College of Science, Jouf University, Sakaka-2014, Saudi Arabia
E-mail: mousagermoush@gmail.com & mogermoush@ju.edu.sa

Abstract

Anisotes trisulcus is a stiff erect shrub with antioxidant and anti-inflammatory activities. This study investigated the protective effect of *A. trisulcus* extract against oxidative stress, inflammation and hypercholesterolemia in high cholesterol diet (HCD)-fed rats. Rats received HCD and *A. trisulcus* extract (100 and 200 mg/kg) for 10 weeks and samples were collected for analysis. Serum cholesterol, triglycerides, LDL-cholesterol and vLDL-cholesterol along with cardiovascular risk indices were significantly increased in HCD-fed rats. HDL-cholesterol was decreased, and pro-inflammatory cytokines and ALT, AST and ALP were increased in serum of HCD-fed rats. Treatment with *A. trisulcus* extract ameliorated these metabolic alterations and decreased hepatic lipid peroxidation levels. In addition, *A. trisulcus* extract enhanced hepatic GSH and antioxidant enzymes in HCD-fed rats. Furthermore, *A. trisulcus* extract decreased the expression of hepatic fatty acid synthase (FAS) and stimulated a trend increase in LDL receptor in HCD-fed rats. In conclusion, this study showed the anti-hypercholesterolemic, antioxidant and anti-inflammatory activities of *A. trisulcus* in HCD-fed rats. *A. trisulcus* ameliorated serum lipids, cardiovascular risk indices, liver function markers and pro-inflammatory cytokines, and hepatic oxidative stress and FAS expression.

Keywords **:** Almodh; LDLR; FAS; Cholesterol; Oxidative stress; Inflammation.

Introduction

Hyperlipidemia/dyslipidemia has been demonstrated as a casual risk factor for atherosclerotic cardiovascular disease (Nordestgaard, 2016). It is a disorder of lipid metabolism and manifested as increased levels of low density lipoprotein (LDL) cholesterol and triacylglycerides (Nordestgaard, 2016). Hyperlipidemia may arise through lifestyle factors such as excessive consumption of foods rich in cholesterol and saturated fats, but is also highly heritable (Ma *et al.*, 2012, Yuan *et al.*, 2007). Hypercholesterolemia is a type of hyperlipidemias that elicits atherosclerosis, chronic inflammation and accumulation of hepatic lipids (steatosis) and hence reduce the ability of the liver to lower circulating lipids (Lee *et al.*, 2017). The role of hypercholesterolemia in eliciting oxidative stress has been demonstrated in different studies (Al-Rejaie *et al.*, 2013; Lee *et al.*, 2017; Olorunnisola *et al.*, 2012). The accumulation of cholesterol in different cells, such as hepatocytes and endothelial cells has been associated with diminished antioxidant defenses and excess production of reactive oxygen species (ROS) (Anila & Vijayalakshmi, 2003; Forstermann, 2008). Excess ROS accumulation can induce inflammation, cell death and metabolic alterations (Jones, 2006; Seifried *et al.*, 2007). Hence, agents that can counteract hypercholesterolemia and other hyperlipidemias and exert antioxidant and anti-inflammatory activities can protect against the metabolic alterations induced by high levels of lipids.

Medicinal plants are rich sources of bioactive compounds that can exert anti-hyperlipidemic effects accompanied with attenuation of oxidative stress and inflammation (Aladaileh *et al.*, 2019b). *Anisotes trisulcus* (family Acanthaceae) is a stiff erect shrub and commonly known as Almodh in Saudi Arabia (El-Shanawany *et al.*, 2011). *A. trisulcus* has been reported to exert multiple activities, including ant-hypertension, anti-bacterial, local anesthetic and hepatoprotective. In addition, this species is used to limit tobacco consumption, suppress appetite and traditionally in the treatment of jaundice, hepatitis, gallstones and other disorders related to the liver (Al-Rehaily *et al.*, 2011; Al-Rehaily *et al.*, 2002; Ali *et al.*, 2001; El-Shanawany *et al.*, 2011). *A. trisulcus* has been demonstrated to be a rich source of phenolic compounds, including vanillic acid, veratric acid, β-sitosterol, stigmasterol, α-amyrin and veratric acid, and its methanolic extract exhibited antioxidant and anti-inflammatory activities (El-Shanawany *et al.*, 2014). However, the beneficial effects of *A. trisulcus* on hypercholesterolemia have not been previously investigated. Therefore, this study evaluated the anti-hypercholesterolemic effect of *A. trisulcus* and its protective antioxidant and anti-inflammatory activities in high cholesterol diet (HCD)-fed rats. Additionally, the ability of *A. trisulcus* to modulate hepatic fatty acid synthase (FAS) and LDL receptor (LDLR) expression in HCD-fed rats was studied.

Materials and Methods

A. trisulcus leaf collection and extraction

The leaves of *A. trisulcus* were collected from the northern are of Saudi Arabis (Sakaka) and were identified and authenticated by an expert taxonomist from the Botany Department, College of Science, Jouf University, KSA. The leaves were washed under running tap water, dried in the shade and then mashed into a powder size and macerated with methanol (80%; v/v) at 4°C for 72 h. The macerated product was filtered, and the obtained supernatant was concentrated using a rotary evaporator to a semi-dry state and then dissolved in distilled water.

Animals and experimental design

Thirty adult male Wistar rats weighing 140-160 g were housed under standard laboratory conditions (12 h light/dark

cycle at 22±25 ºC) with free access to standard rodent pellet diet and water. All experiments and the study protocols were approved by the Ethics Committee of Jouf University (Ethical approval number: 08/01/41). To investigate the anti-hypercholesterolemic effect of *A. trisulcus* extract, the rats were allocated into 5 groups (n=6) as follows: Group I rats fed a normal diet for 10 weeks and served as control, and groups II-V included rats fed a high cholesterol diet (HCD; normal diet supplemented with 2% cholesterol). Groups II and III received 100 and 200 mg/kg *A. trisulcus* extract, respectively, whereas group V received 10 mg/kg simvastatin for 10 weeks. *A. trisulcus* methanolic extract has shown anti-inflammatory effect in rats at dose of 400 mg/kg as reported previously (El-Shanawany *et al.*, 2014). Therefore, two lower doses (100 and 200 mg/kg body weight) were selected in this study.

At the end of the treatment, all groups were fasted overnight, sacrificed under anesthesia and blood samples were collected and left to coagulate to separate serum by centrifugation. The animals were dissected, and liver was existed, washed and homogenized in cold 0.1 M phosphate buffer (pH 7.4). The homogenate was centrifuged at 6000 rpm and the supernatant was collected and stored at -80°C.

Assay of serum liver function parameters

Serum liver parameters transaminases [alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP)] were estimated to assess liver function using Randox diagnostic kits (UK).

Assay of serum lipids and cardiovascular risk indices:

The levels of serum triglycerides (Fossati & Prencipe, 1982), total cholesterol (Allain *et al.*, 1974) and HDL-cholesterol (Burstein *et al.*, 1970) were assayed using Randox (UK) kits. vLDL- and LDL-cholesterol levels were calculated as follows:

vLDL-cholesterol = Triglycerides/5

LDL-cholesterol = Total cholesterol – (HDL-cholesterol + vLDL-cholesterol)

Cardiovascular risk indices (Ross, 1992) and anti-atherogenic index (AAI) (Guido &Joseph, 1992) in all groups were calculated as follows:

Cardiovascular risk index 1 = Total-Cholesterol/HDL-Cholesterol

Cardiovascular risk index 2 = LDL-Cholesterol/HDL-Cholesterol

AAI = HDL-Cholesterol x 100/Total cholesterol - HDL-Cholesterol.

Oxidant/antioxidant status analysis

The assessment of lipid peroxidation (LPO) was based on the amount of formed thiobarbituric acid reactive species (TBARS) according to the described method of Ohkawa *et al.* (1979). The content of reduced glutathione (GSH) was estimated according to the described method by Ellman (1959). The hepatic activity of superoxide dismutase (SOD) and catalase were assayed based on the method of Nishikimi *et al.* (1972) and Aebi (1984), respectively.

Assay of pro-inflammation cytokines

Serum levels of tumor necrosis factor-α (TNF-α), interleukin (IL)-6 and IL-1β were determined using commercial kits (Cusbio, China) according to the manufacturer's protocols.

Quantitative Real Time-PCR (qRT-PCR)

Hepatic total RNA was isolated using TRIzol reagent (Invitrogen, USA) and its quantity was determined using a nanodrop. Samples with A260/A280 higher than 1.7 were immediately reverse-transcribed into cDNA using RevertAid™ H Minus Reverse Transcriptase (Fermentas, Thermo Fisher Scientific Inc., Canada) according to the manufacturer's instructions. For gene expression analysis, qRT-PCR was employed using QuantiFast SYBR Green RT-PCR kit (Qiagen, Hilden, Germany) and the following primers: fatty acid synthase (FAS): F: 5'-CTGGACTCGCTCATGGGTG-3' & R: 5'-CATTTCCTGAAGCTTCCGCAG-3', LDL-receptor (LDL-R): F: 5'-CAGCTCTGTGTGAACCTGGA-3' & R: 5'-TTCTTCAGGTTGGGGATCAG-3', and GAPDH: F: 5'-AACTTTGGCATCGTGGAAGG-3' & R: 5'-TACATTGGGGGTAGGAACAC-3'. qRT-PCR reactions were performed using ViiA™ 7 System (Thermo Fisher Scientific, CA, USA) in duplicates. The transcript number was determined using the $2^{\Delta\Delta}$Ct method (Livak &Schmittgen, 2001).

Statistical analysis

Data were expressed as the mean ± standard error of the mean (SEM) of six rats. Difference between mean values of multiple groups was analyzed using one-way analysis of variance (ANOVA) followed by Tukey's Post hoc test on Graphpad Prism 7. Statistical significance was considered at $P<0.05$.

Results

Effect of *A. trisulcus* on serum lipids

HCD-fed rats showed significantly elevated serum triglycerides ($P<0.001$), total cholesterol ($P<0.001$), LDL-Cholesterol ($P<0.001$) and vLDL-Cholesterol ($P<0.001$) as shown in Figures 1A-D. HDL-Cholesterol was decreased in HCD-fed rats when compared with the control group ($P<0.05$) as shown in Figure 1E. Treatment with *A. trisulcus* extract (100 and 200 mg/kg) and simvastatin decreased serum triglycerides, total cholesterol, LDL-Cholesterol and vLDL-Cholesterol ($P<0.001$) whereas failed to increase HDL-Cholesterol significantly in HCD-fed rats.

Effect of *A. trisulcus* on cardiovascular risk indices

HCD-fed rats exhibited significant increase in cardiovascular risk indices 1 (Fig. 2A) and 2 (Fig. 2B) as compared to the control rats ($P<0.001$). On the other hand, the AAI was decreased significantly in HCD-fed rats ($P<0.001$; Fig. 2C). Treatment with *A. trisulcus* extract and simvastatin decreased the values of cardiovascular risk indices and increased AAI in HCD-fed rats.

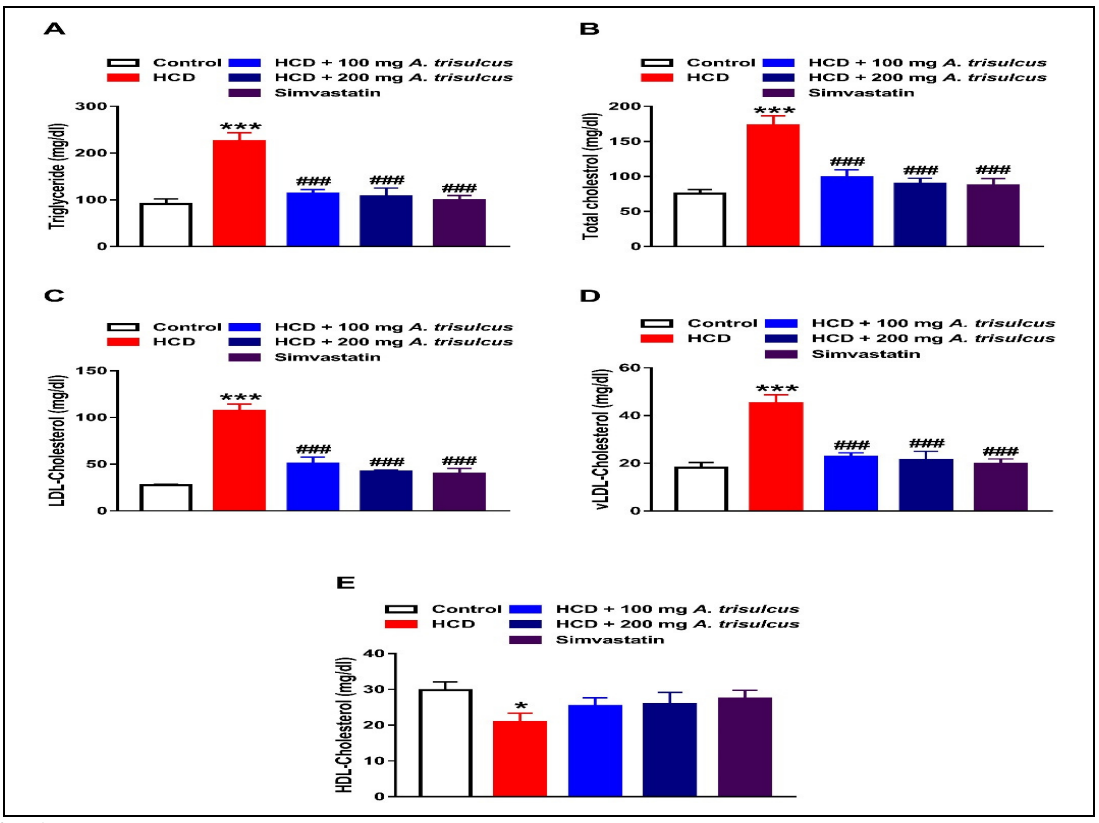

Fig. 1 : Effect of *A. trisulcus* on serum lipids in HCD-fed rats. Data are mean ± SEM (n = 6). *P<0.05 and ***P<0.001 compared to Control. ###P<0.001 compared to HCD.

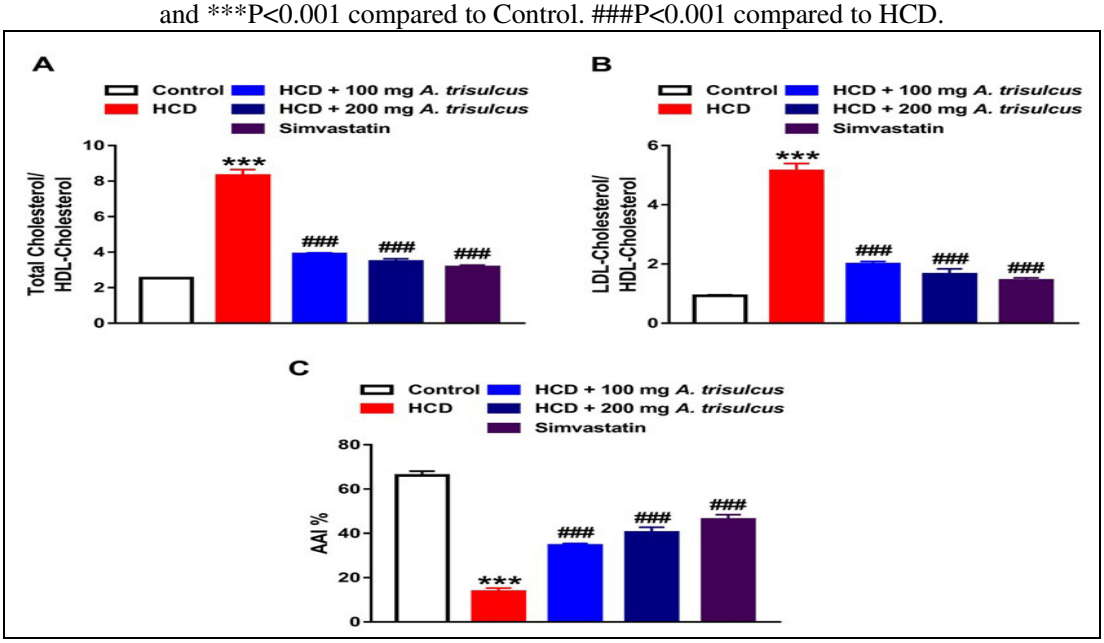

Fig. 2 : Effect of *A. trisulcus* on cardiovascular risk indices and anti-atherogenic index in HCD-fed rats. Data are mean ± SEM (n = 6). ***P<0.001 compared to Control. ###P<0.001 compared to HCD.

Effect of *A. trisulcus* on liver function and serum pro-inflammatory cytokines

ALT, AST and ALP were increased significantly in serum of HCD-fed rats (Fig. 3) as compared to the control group (P<0.001). Both doses of *A. trisulcus* as well as simvastatin decreased serum ALT, AST and ALP significantly in HCD-fed rats.

Similarly, serum TNF-α, IL-6 and IL-1β levels were elevated in HCD-fed rats as compared to the control as represented in Figures 4A-C. In contrast, treatment with *A. trisulcus* as well as simvastatin ameliorated the levels of these inflammatory mediators

Effect of *A. trisulcus* on liver lipid peroxidation and antioxidants

Hepatic TBARS levels were increased significantly (P<0.001) in HCD-fed rats as shown in Figure 5A. On the other hand, hepatic GSH (Fig. 5B), SOD (Fig. 5C) and catalase (Fig. 5D) were decreased in HCD-fed rats. Oral supplementation of *A. trisulcus* (100 and 200 mg/kg) or simvastatin decreased TBARS and increased GSH, SOD and catalase in the liver of HCD-fed rats.

Effect of *A. trisulcus* on LDLR and FAS gene expression

Supplementation of the HCD for 10 weeks exerted non-significant effect on the gene expression levels of LDLR (Fig. 6A) whereas increased hepatic FAS gene expression (Fig. 6B) significantly. Treatment of the HCD-fed rats with *A. trisulcus* (100 and 200 mg/kg) or simvastatin showed a trend increase in LDLR; however non-significant, and decreased FAS gene expression significantly.

.

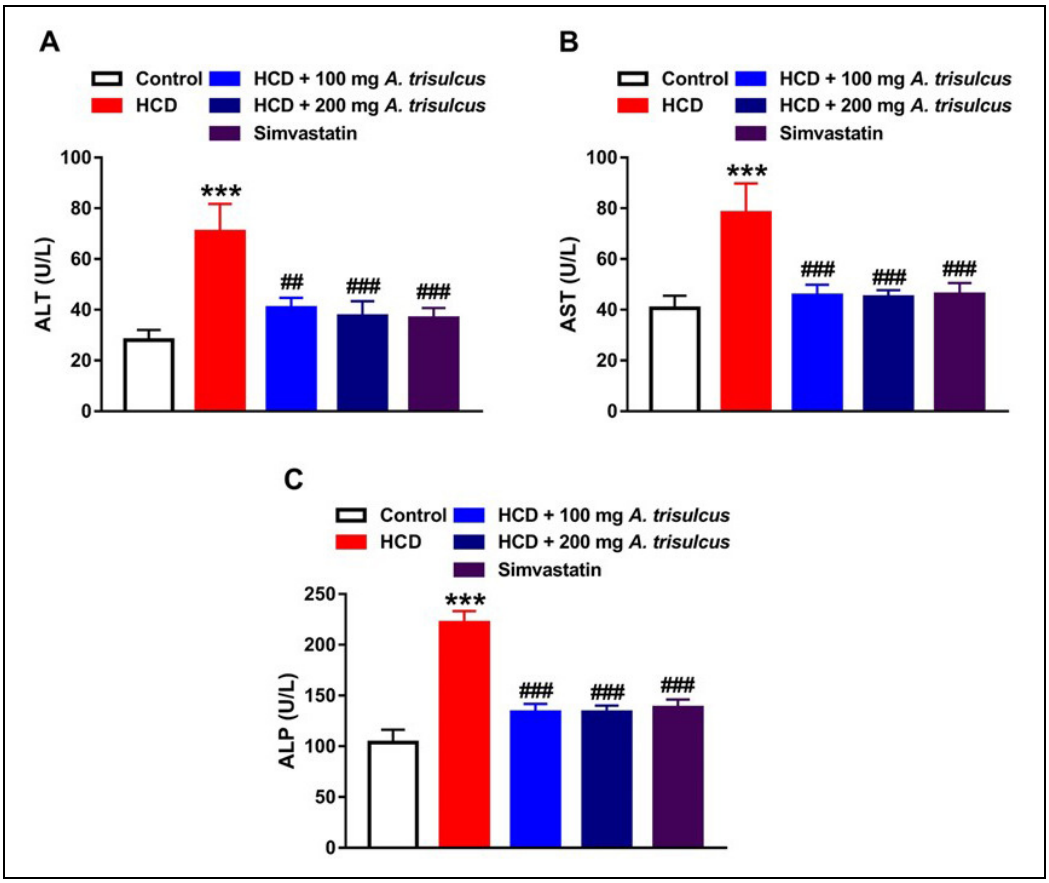

Fig. 3 : Effect of *A. trisulcus* on liver function markers in HCD-fed rats. Data are mean ± SEM (n = 6). ***P<0.001 compared to Control. ###P<0.001 compared to HCD.

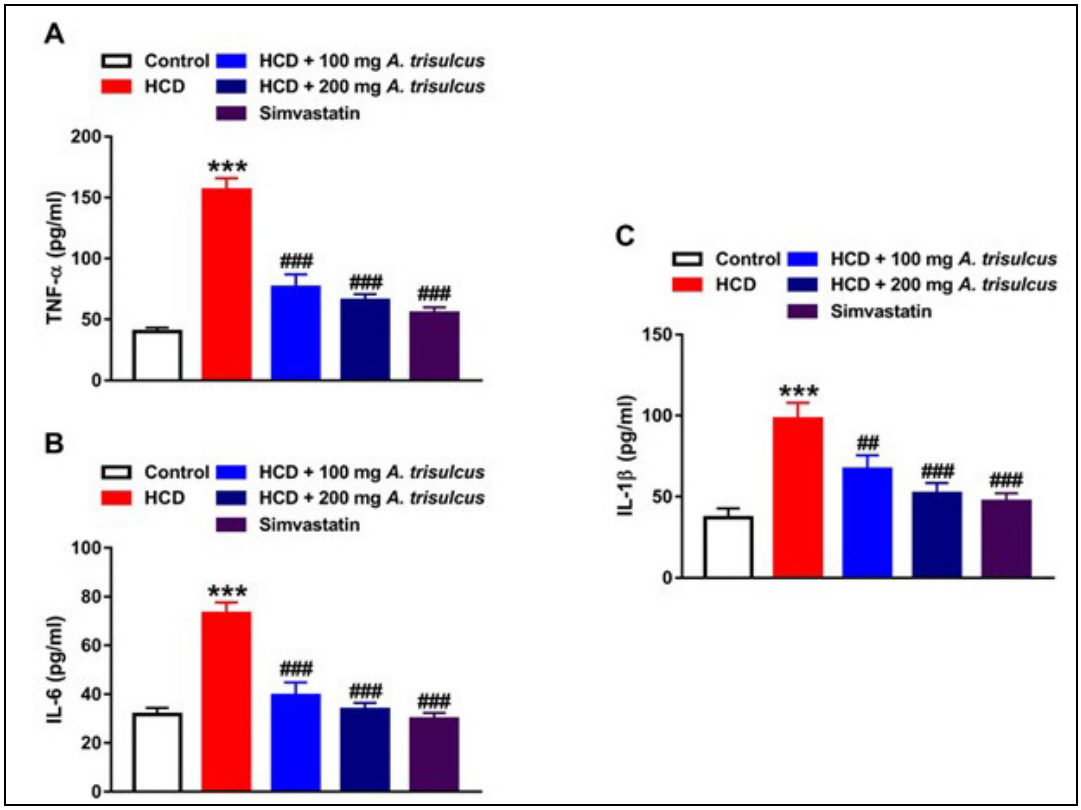

Fig. 4 : Effect of *A. trisulcus* on serum pro-inflammatory cytokines in HCD-fed rats. Data are mean ± SEM (n = 6). *P<0.05 and ***P<0.001 compared to Control. ##P<0.01 and ###P<0.001 compared to HCD.

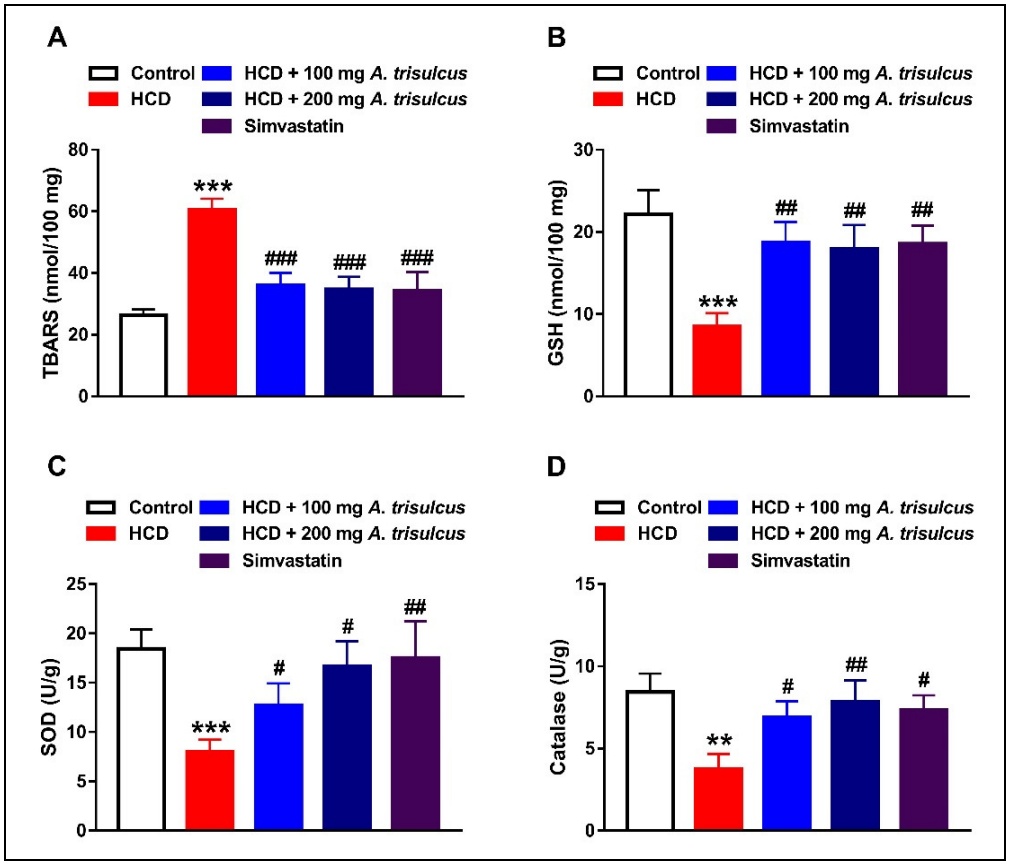

Fig. 5 : Effect of *A. trisulcus* on hepatic lipid peroxidation and antioxidants in HCD-fed rats. Data are mean ± SEM (n = 6). **P<0.01 and ***P<0.001 compared to Control. #P<0.05, ##P<0.01 and ###P<0.001 compared to HCD.

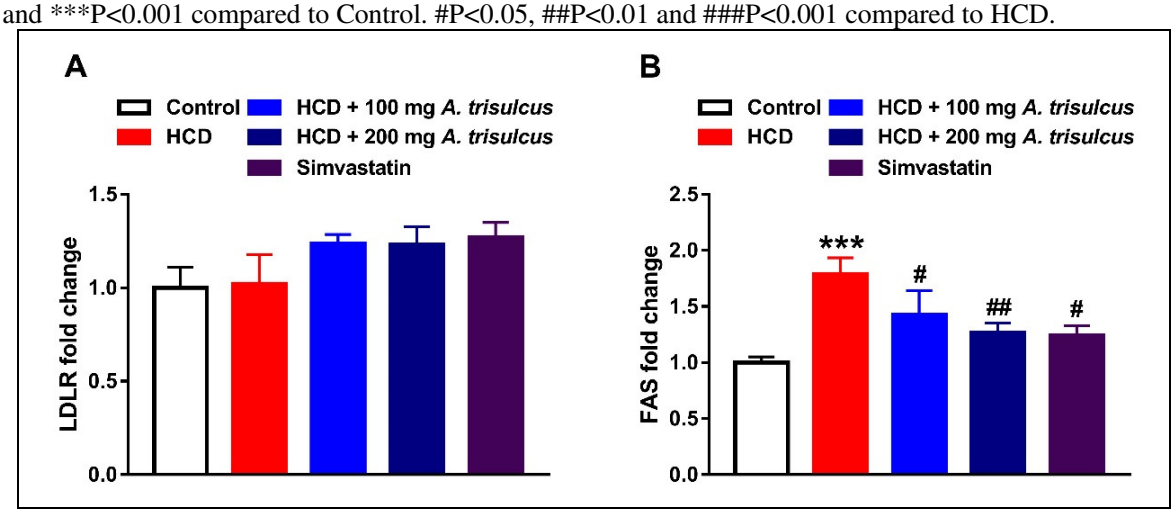

Fig. 6 : Effect of *A. trisulcus* on hepatic LDLR and FAS expression in HCD-fed rats. Data are mean ± SEM (n = 6). ***P<0.001 compared to Control. #P<0.05 and ##P<0.01 compared to HCD.

Discussion

This investigation explored the effects of *A. trisulcus* extract on hypercholesterolemia and its associated oxidative stress and inflammation, and gene expression levels of LDLR and FAS in the liver of rats. Hyperlipidemia is a disorder of lipid metabolism associated with the pathogenesis of atherosclerosis, hypertension, metabolic syndrome, and cardiovascular disease (Al-Rasheed *et al.*, 2018; Al-Rasheed *et al.*, 2017; Attia *et al.*, 2002; Mahmoud *et al.*, 2012; Mahmoud *et al.*, 2017c). In this study, HCD-fed rats exhibited hypercholesterolemia and hypertriglyceridemia manifested by increased serum triglycerides, and total, LDL- and vLDL-cholesterols. Accordingly, previous studies have demonstrated dyslipidemia and hypercholesterolemia in HCD-fed rats (Al-Rejaie *et al.*, 2013; Bin-Jumah, 2018; Lee *et al.*, 2017; Olorunnisola *et al.*, 2012). In addition, feeding a HCD resulted in decreased serum HDL levels and increased the values of cardiovascular risk indices, characteristic features of hypercholesterolemia and cardiovascular diseases (Abd El-Twab *et al.*, 2016; Al-Rasheed *et al.*, 2016; Al-Rasheed *et al.*, 2017; Lee *et al.*, 2017). Interestingly, treatment of the HCD-fed rats with *A. trisulcus* extract resulted in significant amelioration of serum lipids and cardiovascular diseases, demonstrating its potent anti-hyperlipidemic, anti-hypercholesterolemic and cardioprotective effects.

The anti-hyperlipidemic effect of *A. trisulcus* extract could be attributed to its ability to modulate hepatic LDLR and FAS expression. Although it didn't show a significant effect, *A. trisulcus* extract showed a trend increase in LDLR expression in the liver of HCD-fed rats. LDLR is primarily responsible for the absorption of cholesterol by the liver through its mediated endocytosis. Following its absorption, cholesterol is metabolized within the liver and this results in

decreased plasma cholesterol levels (Ma *et al.*, 1986; Yasunobu *et al.*, 1997). Additionally, *A. trisulcus* extract decreased the expression of hepatic FAS significantly in rats fed HCD. Increased expression of hepatic FAS has been previously reported in animal models of rodents fed high fat diet and HCD (Bin-Jumah, 2018; Inoue *et al.*, 2005; Lee *et al.*, 2017). FAS is one of the fatty acid synthesizing enzymes and its suppression is therefore responsible for decreased lipid synthesis.

HCD feeding was also associated with hepatic injury as shown by increased serum levels of ALT, AST and ALP. Hepatic injury occurs as a result of increased hepatic accumulation as a consequence of dyslipidemia (Lee *et al.*, 2017). Accordingly, liver dysfunction has been reported in rats received HCD for 10 weeks (Bin-Jumah, 2018). HCD-induced rats treated with *A. trisulcus* extract showed an improvement in serum ALT, AST and ALP levels, demonstrating its hepatoprotective effect. The ameliorative effect of *A. trisulcus* extract on liver function is a direct consequence of its anti-hypercholesterolemic effect. In addition, the beneficial effect of *A. trisulcus* extract could be attributed to its antioxidant and anti-inflammatory activities. Hypercholesterolemia is implicated in lipid peroxidation and oxidative modification of LDL (Yang *et al.*, 2008). Oxidative injury has been suggested as the mechanism by which hypercholesterolemia provokes cellular death (Ma *et al.*, 1986; Ma *et al.*, 2012). Accordingly, HCD-fed rats exhibited significant increase in hepatic lipid peroxidation and decreased GSH, SOD and catalase as previously demonstrated (Al-Rejaie *et al.*, 2013; Bin-Jumah, 2018; Lee *et al.*, 2017; Olorunnisola *et al.*, 2012). In the same context, hyperlipidemia has been shown to a risk factor for eliciting oxidative stress, cell death and tissue injury (Huisamen *et al.*, 2012; Vincent *et al.*, 2001). *A. trisulcus* extract prevented oxidative stress as shown by decreased TBARS and increased antioxidants. These findings pointed to the antioxidant activity of *A. trisulcus*. By using in vitro DPPH-radical scavenging assay, the antioxidant activity of *A. trisulcus* has been previously shown (El-Shanawany *et al.*, 2014).

Besides its antioxidant effect, *A. trisulcus* has demonstrated potent anti-inflammatory effects in carrageenan-induced rat hind paw edema (El-Shanawany *et al.*, 2014). In this investigation, *A. trisulcus* ameliorated serum levels of the pro-inflammatory cytokines, TNF-α, IL-6 and IL-1β, in HCD-fed rats. the induction of hypercholesterolemia in rats has been associated with inflammatory responses marked by increased serum cytokines and c-reactive protein (Bin-Jumah, 2018). This inflammatory response could be explained by the hypercholesterolemia-induced oxidative stress which causes inflammation by activating nuclear factor-kappaB (NF-κB) and increasing the production of proinflammatory cytokines, including TNF-α and IL-6. In addition, oxidative modification of LDL particles can induce the expression of adhesive molecules and lead to secretion of cytokine (Rocha & Libby, 2009).

The antioxidant, anti-inflammatory and anti-hyperlipidemic effects of *A. trisulcus* are attributed to its rich content of bioactive compounds, particularly phenolics. Phenolic compounds possess multiple effects, including antioxidant, anti-inflammatory, hepatoprotective, anti-hyperlipidemic, and anti-diabetic (Al-Dossari *et al.*, 2019; Aladaileh *et al.*, 2019a; Alhusaini *et al.*, 2019; Althunibat *et al.*, 2019; Kamel *et al.*, 2016; Mahmoud, 2012, 2013; Mahmoud *et al.*, 2017a; Mahmoud *et al.*, 2017b). Phytochemical analysis of the methanolic extract *A. trisulcus* revealed the presence of vanillic acid, veratric acid, α-amyrin and many other compounds with antioxidant and anti-inflammatory activities (El-Shanawany *et al.*, 2014). For instance, vanillic acid has shown protective effects against high fat diet (HFD)-induced hyperlipidemia and inflammation in rats (Chang *et al.*, 2015), and activates thermogenesis in brown and white adipose tissue of HFD-fed mice (Han *et al.*, 2018). Vanillic acid has also been reported to attenuate obesity via activation of the AMPK pathway as well as thermogenic factors both in vivo and in vitro (Jung *et al.*, 2018).

Conclusion

The findings of this study show for the first time the anti-hypercholesterolemic, antioxidant, anti-inflammatory and hepatoprotective activities of *A. trisulcus* in HCD-fed rats. *A. trisulcus* ameliorated serum lipids and cardiovascular risk indices. Treatment of the HCD-fed rats exhibited significant improvement of liver function markers, serum pro-inflammatory cytokines, lipid peroxidation and antioxidant defenses. The lipid-lowering effect of *A. trisulcus* is mediated, at least in part, by its ability to modulate LDLR and FAS expression. However, investigations are needed to determine the exact mechanisms underlying the lipid-lowering effect of *A. trisulcus*.

Conflict of interest

No conflict of interest

References

Abd El-Twab, S.M.; Mohamed, H.M. and Mahmoud, A.M. (2016). Taurine and pioglitazone attenuate diabetes-induced testicular damage by abrogation of oxidative stress and up-regulation of the pituitary-gonadal axis. Can J Physiol Pharmacol, 94: 651-61.

Aebi, H. (1984). Catalase in vitro. Methods Enzymol, 105: 121-6.

Al-Dossari, M.H.; Fadda, L.M.; Attia, H.A.; Hasan, I.H. and Mahmoud, A.M. (2019). Curcumin and Selenium Prevent Lipopolysaccharide/Diclofenac-Induced Liver Injury by Suppressing Inflammation and Oxidative Stress. Biological trace element research.

Al-Rasheed, N.M.; Al-Rasheed, N.M.; Hasan, I.H.; Al-Amin, M.A.; Al-Ajmi, H.N.; Mahmoud, A.M. (2016). Sitagliptin attenuates cardiomyopathy by modulating the JAK/STAT signaling pathway in experimental diabetic rats. Drug design, development and therapy, 10: 2095-107.

Al-Rasheed, N.M.; Al-Rasheed, N.M.; Hasan, I.H.; Alamin, M.; Al-Ajmi, H.N.; Mohammad, R.A. and Mahmoud, A.M. (2017). Simvastatin ameliorates diabetic cardiomyopathy by attenuating oxidative stress and inflammation in rats. Oxid Med Cell Longev 2017, Article ID: 1092015

Al-Rasheed, N.M.; Al-Rasheed, N.M.; Bassiouni, Y.A.; Hasan, I.H.; Al-Amin, M.A.; Al-Ajmi, H.N. and Mahmoud, A.M. (2018). Simvastatin ameliorates diabetic nephropathy by attenuating oxidative stress and apoptosis in a rat model of streptozotocin-induced type

1 diabetes. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie, 105: 290-298.

Al-Rehaily, A.J.; El-Sayed, K.A.; Al-Said, M.S. and Ahmed, B. (2002). Trisulcusine: A novel spiro quinazoline alkaloid from *Anisotes trisulcus*. Indian Journal of Chemistry - Section B Organic and Medicinal Chemistry, 41: 2385-2389.

Al-Rehaily, A.J.; Al-Said, M.S. and Eltahir, K.E.H. (2011). Peganine isolated from *Anisotes trisulcus* as a smoking deterrent and anorexigenic agent. African Journal of Pharmacy and Pharmacology, 5: 1342-1348.

Al-Rejaie, S.S.; Aleisa, A.M.; Sayed-Ahmed, M.M.; Al-Shabanah, O.A.; Abuohashish, H.M.; Ahmed, M.M.; Al-Hosaini, K.A. and Hafez, M.M. (2013). Protective effect of rutin on the antioxidant genes expression in hypercholestrolemic male Westar rat. BMC complementary and alternative medicine, 13: 136.

Aladaileh, S.H.; Abukhalil, M.H.; Saghir, S.A.M.; Hanieh, H.; Alfwuaires, M.A.; Almaiman, A.A.; Bin-Jumah, M. and Mahmoud, A.M. (2019a). Galangin Activates Nrf2 Signaling and Attenuates Oxidative Damage, Inflammation, and Apoptosis in a Rat Model of Cyclophosphamide-Induced Hepatotoxicity. Biomolecules, 9: 346.

Aladaileh, S.H.; Saghir, S.A.M.; Murugesu, K.; Sadikun, A.; Ahmad, A.; Kaur, G.; Mahmoud, A.M. and Murugaiyah, V. (2019b). Antihyperlipidemic and Antioxidant Effects of *Averrhoa carambola* Extract in High-Fat Diet-Fed Rats. Biomedicines, 7: 72.

Alhusaini, A.; Fadda, L.; Hasan, I.H.; Zakaria, E.; Alenazi, A.M. and Mahmoud, A.M. (2019). Curcumin ameliorates Lead-Induced Hepatotoxicity by Suppressing Oxidative Stress and Inflammation, and Modulating Akt/GSK-3beta Signaling Pathway. Biomolecules, 9: 703.

Ali, N.A.A.; Jülich, W.D.; Kusnick, C. and Lindequist, U. (2001). Screening of Yemeni medicinal plants for antibacterial and cytotoxic activities. Journal of Ethnopharmacology, 74; 173-179.

Allain, C.C.; Poon, L.S.; Chan, C.S.; Richmond, W. and Fu, P.C. (1974). Enzymatic determination of total serum cholesterol. Clinical chemistry, 20: 470-5.

Althunibat, O.Y.; Al-Hroob, A.M.; Abukhalil, M.H.; Germoush, M.O.; Bin-Jumah, M. and Mahmoud, A.M. (2019). Fisetin ameliorates oxidative stress, inflammation and apoptosis in diabetic cardiomyopathy. Life sciences, 221: 83-92.

Anila, L. and Vijayalakshmi, N.R. (2003). Antioxidant action of flavonoids from *Mangifera indica* and *Emblica officinalis* in hypercholesterolemic rats. Food Chemistry, 83: 569-574.

Attia, D.M.; Ni, Z.N.; Boer, P.; Attia, M.A.; Goldschmeding, R.; Koomans, H.A.; Vaziri, N.D. and Joles, J.A. (2002): Proteinuria is preceded by decreased nitric oxide synthesis and prevented by a NO donor in cholesterol-fed rats. Kidney international, 61: 1776-87.

Bin-Jumah, M. (2018). Monolluma quadrangular protects against oxidative stress and modulates LDL receptor and fatty acid synthase gene expression in hypercholesterolemic rats. Oxidative medicine and cellular longevity, 2018: 3914384.

Burstein, M.; Scholnick, H.R. and Morfin, R. (1970). Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. J Lipid Res., 11: 583-95.

Chang, W-C.; Wu, JS-B.; Chen, C-W.; Kuo, P-L.; Chien, H-M.; Wang, Y-T. and Shen, S-C. (2015). Protective Effect of Vanillic Acid against Hyperinsulinemia, Hyperglycemia and Hyperlipidemia via Alleviating Hepatic Insulin Resistance and Inflammation in High-Fat Diet (HFD)-Fed Rats. Nutrients, 7: 9946-9959.

El-Shanawany, M.A.; Sayed, H.M.; Ibrahim, S.R.M. and Fayed, M.A.A. (2011). 5-Hydroxy vasentine, a new pyrroloquinazoline alkaloid from Anisotes trisulcus (Forssk.) Nees. J. Nat. Prod. Plant Res., 1: 80-85.

El-Shanawany, M.A.; Sayed, H.M.; Ibrahim, S.R.M. and Fayed, M.A.A. (2014). Chemical constituents, anti-inflammatory, and antioxidant activities of Anisotes trisulcus. Bulletin of Faculty of Pharmacy, Cairo University, 52: 9-14.

Ellman, G.L. (1959). Tissue sulfhydryl groups. Arch Biochem Biophys, 82: 70-7.

Forstermann, U. (2008). Oxidative stress in vascular disease: causes, defense mechanisms and potential therapies. Nature clinical practice. Cardiovascular medicine, 5: 338-49.

Fossati, P. and Prencipe, L. (1982). Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clinical chemistry, 28: 2077-80.

Guido, S. and Joseph, T. (1992). Effect of chemically different calcium antagonists on lipid profile in rats fed on a high fat diet. Indian journal of experimental biology, 30: 292-4.

Han, X.; Guo, J.; You, Y.; Yin, M.; Liang, J.; Ren, C.; Zhan, J. and Huang, W. (2018). Vanillic acid activates thermogenesis in brown and white adipose tissue. Food & Function, 9: 4366-4375.

Huisamen, B.; Dietrich, D.; Bezuidenhout, N.; Lopes, J.; Flepisi, B.; Blackhurst, D. and Lochner, A. (2012). Early cardiovascular changes occurring in diet-induced, obese insulin-resistant rats. Molecular and cellular biochemistry, 368: 37-45.

Inoue, M.; Ohtake, T.; Motomura, W.; Takahashi, N.; Hosoki, Y.; Miyoshi, S.; Suzuki, Y.; Saito, H.; Kohgo, Y. and Okumura, T. (2005). Increased expression of PPARgamma in high fat diet-induced liver steatosis in mice. Biochem Biophys Res Commun, 336: 215-22.

Jones, D.P. (2006). Redefining oxidative stress. Antioxidants & redox signaling, 8: 1865-79.

Jung, Y.; Park, J.; Kim, H-L.; Sim, J-E.; Youn, D-H.; Kang, J.; Lim, S.; Jeong, M-Y.; Yang, W.M.; Lee, S-G.; Ahn, K.S. and Um, J-Y. (2018). Vanillic acid attenuates obesity via activation of the AMPK pathway and thermogenic factors in vivo and in vitro. The FASEB Journal, 32: 1388-1402.

Kamel, E.M.; Mahmoud, A.M.; Ahmed, S.A. and Lamsabhi, A.M. (2016). A phytochemical and computational study on flavonoids isolated from *Trifolium resupinatum* L. and their novel hepatoprotective activity. Food Funct. 2016 Apr; 7(4): 2094-106.

Lee, K.S.; Chun, S.Y.; Kwon, Y.S.; Kim, S. and Nam, K.S. (2017). Deep sea water improves hypercholesterolemia and hepatic lipid accumulation through the regulation of hepatic lipid metabolic gene expression. Molecular medicine reports, 15: 2814-2822.

Livak, K.J. and Schmittgen, T.D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods, 25: 402-8.

Ma, P.T.; Gil, G.; Sudhof, T.C.; Bilheimer, D.W.; Goldstein, J.L. and Brown, M.S. (1986). Mevinolin, an inhibitor of cholesterol synthesis, induces mRNA for low density lipoprotein receptor in livers of hamsters and rabbits. Proceedings of the National Academy of Sciences of the United States of America, 83: 8370-4.

Ma, Y.; Wang, W.; Zhang, J.; Lu, Y.; Wu, W.; Yan, H. and Wang, Y. (2012). Hyperlipidemia and atherosclerotic lesion development in Ldlr-deficient mice on a long-term high-fat diet. PloS one 7: e35835.

Mahmoud, A.M. (2012). Influence of rutin on biochemical alterations in hyperammonemia in rats. Exp Toxicol Pathol. 2012 Nov; 64(7-8): 783-9.

Mahmoud, A.M.; Ashour, M.B.; Abdel-Moneim, A. and Ahmed, O.M. (2012). Hesperidin and naringin attenuate hyperglycemia-mediated oxidative stress and proinflammatory cytokine production in high fat fed/streptozotocin-induced type 2 diabetic rats. Journal of Diabetes and its Complications, 26: 483-490.

Mahmoud, A.M. (2013). Hematological alterations in diabetic rats - Role of adipocytokines and effect of citrus flavonoids. EXCLI J, 12: 647-57.

Mahmoud, A.M.; Abd El-Twab, S.M. and Abdel-Reheim, E.S. (2017a). Consumption of polyphenol-rich Morus alba leaves extract attenuates early diabetic retinopathy: the underlying mechanism. European journal of nutrition, 56: 1671-1684.

Mahmoud, A.M.; Mohammed, H.M.; Khadrawy, S.M. and Galaly, S.R. (2017b). Hesperidin protects against chemically induced hepatocarcinogenesis via modulation of Nrf2/ARE/HO-1, PPARgamma and TGF-beta1/Smad3 signaling, and amelioration of oxidative stress and inflammation. Chemico-biological interactions, 277: 146-158.

Mahmoud, A.M.; Wilkinson, F.L.; Jones, A.M.; Wilkinson, J.A.; Romero, M.; Duarte, J. and Alexander, M.Y. (2017c). A novel role for small molecule glycomimetics in the protection against lipid-induced endothelial dysfunction: Involvement of Akt/eNOS and Nrf2/ARE signaling. Biochim Biophys Acta, 1861: 3311-3322.

Nishikimi, M.; Appaji, N. and Yagi, K. (1972). The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem Biophys Res Commun, 46: 849-54.

Nordestgaard, B.G. (2016). Triglyceride-Rich Lipoproteins and Atherosclerotic Cardiovascular Disease: New Insights From Epidemiology, Genetics, and Biology. Circulation research, 118: 547-63.

Ohkawa, H.; Ohishi, N. and Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem, 95: 351-8.

Olorunnisola, O.S.; Bradley, G. and Afolayan, A.J. (2012). Protective effect of *T. violacea* rhizome extract against hypercholesterolemia-induced oxidative stress in Wistar rats. Molecules (Basel, Switzerland), 17: 6033-45.

Rocha, V.Z. and Libby, P. (2009). Obesity, inflammation, and atherosclerosis. Nature reviews. Cardiology, 6: 399-409.

Ross, R. (1992). The Pathogenesis of Atherosclerosis. In: Braunwald E (Editor), Heart Disease: A Textbood of Cardiovascular Medicine. PA: WB Saunders, Philadelphia, 1106-1124.

Seifried, H.E.; Anderson, D.E.; Fisher, E.I. and Milner, J.A. (2007). A review of the interaction among dietary antioxidants and reactive oxygen species. The Journal of nutritional biochemistry, 18: 567-79.

Vincent, H.K.; Powers, S.K.; Dirks, A.J. and Scarpace, P.J. (2001). Mechanism for obesity-induced increase in myocardial lipid peroxidation. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity, 25: 378-88.

Yang, R.L.; Shi, Y.H.; Hao, G.; Li, W. and Le, G.W. (2008). Increasing Oxidative Stress with Progressive Hyperlipidemia in Human: Relation between Malondialdehyde and Atherogenic Index. Journal of clinical biochemistry and nutrition, 43: 154-8.

Yasunobu, Y.; Hayashi, K.; Shingu, T.; Nomura, K.; Ohkura, Y.; Tanaka, K.; Kuga, Y.; Nomura, S.; Ohtani, H.; Nishimura, T.; Matsuura, H. and Kajiyama, G. (1997). Reduction of plasma cholesterol levels and induction of hepatic LDL receptor by cerivastatin sodium (CAS 143201-11-0, BAY w 6228), a new inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase, in dogs. Cardiovascular drugs and therapy, 11: 567-74.

Yuan, G.; Al-Shali, K.Z. and Hegele, R.A. (2007). Hypertriglyceridemia: its etiology, effects and treatment. CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne, 176: 1113-20.