

Evaluation of Antihyperlipidemic Activity of Eugenol in Triton Induced Hyperlipidemia in Rats

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Abstract: *Hyperlipidemia is the greatest risk factor of coronary heart disease. Currently available hypolipidemic drugs have been associated with number of side effects. Herbal treatment for hyperlipidemia has little side effects and is relatively cheap and locally available. This study investigated the antihyperlipidemic effect of eugenol in tritonwr1339 induced hyperlipidemia in rats. The hyperlipidemic rats were randomized into six groups and administered groups, group 1 saline (0.9 NaCl), group 2 sesame oil, group 3 eugenol (250mg/kg), group 4 atorvastatin (40 mg/kg), group 5 atorvastatin and eugenol and group 6 hyperlipidemic pretreated with eugenol. The animals were treated orally once daily for seven days. The results clearly demonstrated that eugenol as well as atorvastatin produced highly significant decline in lipid profile. Combination of eugenol and atorvastatin showed highly significant reduction in lipid profile. Eugenol revealed highly significant reduction in lipid profile when administered seven days before hyperlipidemia induction. Administration of eugenol showed highly significant increase in SOD level in liver while significant elevation in kidney tissue. Eugenol produced highly significant elevation in CAT level in liver and kidney tissues. Atorvastatin showed no significant change in SOD level while significant elevation on CAT level in liver and highly significant increase in kidney tissue. Combination of eugenol and atorvastatin revealed highly significant increase in SOD and CAT in both liver and kidney tissues. Administration of eugenol one week before hyperlipidemia induction showed no significant change in SOD in liver and kidney tissues and CAT in liver but there was high significant increase in the activity of CAT in kidney. These results indicate that combination of atorvastatin and eugenol improves lipid profile and the activities of SOD and CAT in both liver and kidney tissues. To overcome hyperlipidemia problems and its side effects, eugenol might provide a useful source of new oral hypolipidemic and antioxidant compound for the development of pharmaceuticals entities or as dietary adjunct to existing therapies.*

Keywords: *Hyperlipidemia, Triglycerides, lipoproteins, eugenol, clove oil, antioxidants*

1. INTRODUCTION

Hyperlipidemia has been ranked as one of the greatest risk factors contributing to the prevalence and severity of coronary heart diseases. World Health Organization reports that high blood cholesterol contributes to approximately 56% cases of cardiovascular diseases worldwide and causes about 4.4 million deaths each year. Cardiovascular diseases including coronary heart disease and stroke are the leading cause of mortality, accounting for nearly 50% of all deaths in the Western developed world (Krishna et al., 2012). Medications from major classes of drugs have been reported to treat people with detrimental lipid levels that include statins, nicotinic acid derivatives, fibric acid derivatives, bile acid binding resins and cholesterol absorption inhibitors. Control of cholesterol levels through therapeutic drugs as statins have sufficiently reduced the risk for developing atherosclerosis and associated cardiovascular diseases. Adverse effects associated with statins such as myopathy, liver damages and potential drug-drug interactions have been reported. Therefore, development of additional therapies for controlling cholesterol levels is warranted especially for those with better safety profile (Ruitang, 2009). There are evidences that free radicals induced oxidation of lipoproteins which is an important event in the pathogenesis of atherosclerosis.

Hence the choice of ideal hypolipidemic drugs would depend on their antioxidant potential as well (Naresh et al., 2010). Therefore, on the above stated facts there is a need to have a drug having the dual property of lowering lipid level and antioxidant activities together with minimal side effects and so the search for compounds from natural sources for reduction of serum cholesterol and reduction of hypercholesterolemic atherosclerosis is necessary (Ola et al., 2011). Herbal remedies or food supplements have increasingly become attractive alternatives to prevent or treat hypercholesterolemia,

especially for those with cholesterol at the borderline levels (Ruitang, 2009). In this study we evaluated the lipid lowering activity and antioxidant potential of eugenol in triton induced hyperlipidemic rats and compared it with atorvastatin. Furthermore, evaluation of combining effects of eugenol and atorvastatin.

2. MATERIAL AND METHODS

2.1 Animals

Male adult albino rats weighing 150-200 grams at the age of 3.0-4.0 months have been used. Animals were obtained from the animal house, Faculty of Medicine, Assiut University and were housed in animal place with room temperature being maintained at 25 ± 2 °C. Animals were fed on a commercial pellet diet and kept under normal light/dark cycle. Animals were given a free access for food and water up to their use. The experimental protocol was approved by the Institutional Animal Care and Use Committee of Sohag University, School of Medicine.

2.2 Chemicals and solutions

Eugenol

(Sigma Aldrich Company, England). Eugenol was pure oily solution, bottle contain 100 ml and freshly diluted with sesame oil (Abraham, 2001).

Atorvastatin calcium

(Sigma Aldrich Company, England). It was available in as a powder, 5 gm. in bottle and has been freshly suspended in distilled water before administration (Mohsen et al., 2010).

Triton wr1339 (tyloxapol)

(Sigma Aldrich Company, England). A nonionic liquid polymer of the alkyl aryl polyether alcohol type, bottle contains 10gm oily solution. It was dissolved in 0.9% saline (Deepak et al., 2011).

Sesame oil

(Nile Company for pharmaceuticals, Cairo, Egypt). It has been used for dilution of eugenol, 1ml/rat/day (Pourgholami et al., 1998).

Diagnostic commercial kits

(Human Gesellschaft for Biochemica and Diagnostic mbH. Germany). They have been used for measuring lipid profile. (Biodiagnostic Company Pharmaceutical industries. Egypt). They were used for measuring oxidative stress markers Super Oxide Dismutase (SOD) and Catalase (CAT).

Induction of hyperlipidemia

Hyperlipidemia was induced in overnight (16 hours) fasted rats by single intra-peritoneal injection of triton wr 1339 (tyloxapol) (350 mg/kg body weight) dissolved in 0.5ml saline (Deepak et al., 2011). Control rats received the same amount of saline. Development of hyperlipidemia in rats was confirmed by measuring total blood cholesterol (blood samples from retro-orbital sinus, 0.5ml/each rat), 48 hours after triton administration (Deepak et al., 2011).

3. TREATMENT

The animals were randomly divided into six groups. Each group comprised six rats. Saline, sesame oil, eugenol (250 mg/kg), atorvastatin (40mg/ kg) (Patel et al., 2010), eugenol and atorvastatin combination were administered once daily for seven days by oral gavage tube after induction of hyperlipidemia. Eugenol prophylaxis group was administered once daily orally for one week before hyperlipidemia induction. The animals were treated as follow:

Group 1: Non-hyperlipidemic + saline (0.5 ml)

Group 2: Hyperlipidemic + sesame oil (1.0 ml/rat)

Group 3: Hyperlipidemic + eugenol (250 mg/kg) (Abraham, 2001).

Group 4: Hyperlipidemic + atorvastatin (40 mg/kg)

Group 5: Hyperlipidemic + eugenol + atorvastatin

Group 6: Eugenol (250 mg/kg) prophylaxis

3.1 Blood Sampling and Serum Preparation

At the end of treatment period, the investigated animals of all groups were fasted for at least 16 hours and sacrificed by decapitation. Blood samples were collected in centrifuge tubes. Serum was separated and stored quickly at -80°C for biochemical analysis.

3.2 Biochemical Analysis

Serum samples collected were used for measuring total cholesterol as described by Richmond (1973), triglycerides by Stein (1987), HDL as described by Friedewald et al., (1972), Serum LDL and VLDL cholesterol was determined by Friedewald formula, $LDL = \text{Total cholesterol} - (\text{HDL} + (\text{TG}/5))$, $VLDL = \text{TG}/5$ (Friedewald et al., 1972).

3.3 Tissue Sampling

Liver and kidney specimens were isolated and perfused immediately with a PBS (phosphate buffered saline) solution, PH 7.4 containing 0.16 mg/ml heparin to remove any red blood cells and clots to be ready for homogenization and measuring antioxidant enzymes.

3.4 Assay of Oxidative Stress

Tissue SOD was determined by a colorimetric method as described by Nishikimi et al., (1972) using a diagnostic kit supplied by Biodiagnostic Company (Cairo-Egypt). Tissue catalase was determined by a colorimetric method as described by Aebi (1984) using diagnostic kit supplied by Biodiagnostic Company (Cairo-Egypt).

3.5 Statistical Analysis

The commercially available statistical analysis software (IBM-SPSS version 19.0 for windows; IBM Inc) was used for data analysis. Degree of variability in results was expressed as the mean ± standard deviation (SD) of the means. ANOVA test followed by Post Hoc Test was used for statistical analysis. The level of significance between groups were considered significant (*) at $p < 0.05$ and highly significant (**) at $p < 0.01$.

4. RESULTS

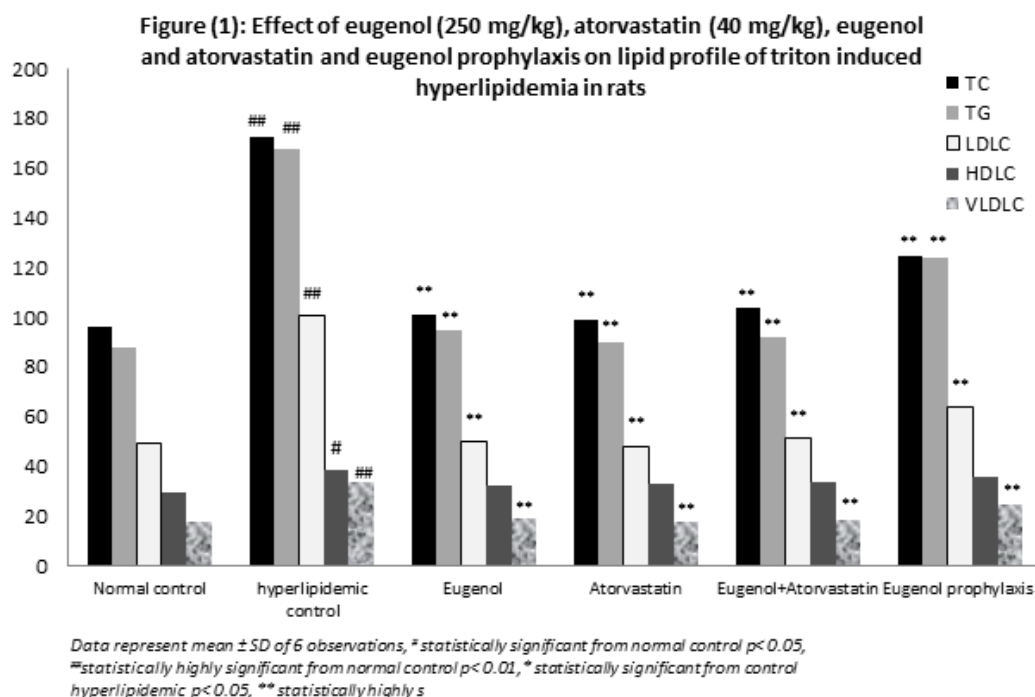
4.1 Lipid Profile

Table (1) summarize the results of lipid profile in different groups of animals at the end of one week treatment with eugenol (250 mg/kg), atorvastatin (40mg/kg), combined eugenol and atorvastatin and eugenol prophylaxis. The results showed that triton wr 1339 (350 mg/kg) successfully induced hyperlipidemia among experimental rats as shown in table (1) and fig. (1). Lipid profile of hyperlipidemic control rats was significantly higher than corresponding control normal rats. Results showed that high significant reduction in total cholesterol 41.29%, triglyceride 43.28%, LDL cholesterol 50.66%, and VLDL 43.28% after eugenol administration. Also atorvastatin revealed high significant reduction 42.74% in TC, 46.26% in TG, 52.31% in LDLC and 46.26% in VLDL. Combined eugenol and atorvastatin administration to hyperlipidemic rats showed highly significant reduction in TC 39.94%, TG 42.27%, LDLC 49.01% and 44.26% in VLDL. Results revealed that administration of eugenol for one week before induction of hyperlipidemia highly significant reduce the elevation in TC by 27.85%, in TG by 25.86%, in LDLC by 36.75% and in VLDL by 25.88%. There was no significant change in HDL level.

Table 1. Effect of eugenol (250 mg/kg), atorvastatin (40 mg/kg), eugenol+atorvastatin and eugenol prophylaxis on lipid profile of triton induced hyperlipidemia in rats.

parameters	Normal control	Hyperlipidemic control	Hyperlipidemic + eugenol	Hyperlipidemic +atorvastatin	Hyperlipidemic +eugenol and atorvastatin	Hyperlipidemic pretreated with eugenol
TC	96.17 ± 5.87	172.33 ^{###} ± 12.77	101.17 ^{**} ± 4.35	98.67 ^{**} ± 4.92	103.50 ^{**} ± 5.95	124.33 ^{**} ± 9.52
TG	87.5 ± 9.35	167.5 ^{###} ± 9.35	95.00 ^{**} ± 7.07	90.00 ^{**} ± 7.07	91.67 ^{**} ± 8.16	124.17 ^{**} ± 7.36
LDL	49.0 ± 6.19	100.67 ^{###} ± 8.33	49.67 ^{**} ± 1.86	48.00 ^{**} ± 2.68	51.33 ^{**} ± 6.65	63.67 ^{**} ± 7.89
HDL	29.67 ± 2.8	38.17 [#] ± 5.34	32.50 ± 3.27	32.67 ± 3.38	33.50 ± 4.59	35.83 ± 3.97
VLDL	17.5 ± 1.87	33.50 ^{###} ± 1.87	19.00 ^{**} ± 1.41	18.00 ^{**} ± 1.41	18.67 ^{**} ± 2.16	24.83 ^{**} ± 1.47

Data represent mean \pm SD of 6 observations, # statistically significant from normal control $p < 0.05$, ## statistically highly significant from normal control $p < 0.01$, * statistically significant from control hyperlipidemic $p < 0.05$, ** statistically highly significant from control hyperlipidemic $p < 0.01$.



4.2 Oxidative Stress

Table (2) shows the results of SOD and CAT activities in liver and kidney tissues in different groups of animals at the end of one week treatment with eugenol (250 mg/kg), atorvastatin (40mg/kg), combined eugenol and atorvastatin and eugenol prophylaxis. There was highly significant reduction in the activity of SOD in liver and kidney tissues in triton induced hyperlipidemic rats from 346 and 2236.4 u/gm in normal control to 75.19 and 689.2 u/gm. in triton induced hyperlipidemic rats respectively as shown in table (2) and figure (2).

There was significant reduction in CAT activity in the liver tissues of triton induced hyperlipidemic rats from 7.44 ± 3.55 u/gm in normal rats to 2.33 ± 1.06 u/gm in triton induced hyperlipidemic rats, while there was highly significant reduction of CAT activity in the kidney tissues of triton induced hyperlipidemic rats from 20.9 ± 2.58 u/gm in normal rats to 8.14 ± 1.53 u/gm. in triton induced hyperlipidemic rats as shown in table (2) and figure (3).

Administration of eugenol showed highly significant increase in the SOD activity of liver tissues from 75.19 ± 8.87 u/gm in triton induced hyperlipidemic rats to 229.16 ± 44.7 u/gm in eugenol treated hyperlipidemic rats. There was significant increase in the activity of SOD in the kidney tissues from 689.2 ± 46.19 u/gm in triton induced hyperlipidemic rats to 1184.2 ± 306 u/gm in eugenol treated hyperlipidemic rats as shown in table (2) and figure (2).

Eugenol administration produced highly significant elevation in the activity of CAT in liver tissues of hyperlipidemic rats from 2.33 ± 1.06 u/gm in control hyperlipidemic rats to 8.17 ± 2.44 u/gm in hyperlipidemic rats treated with eugenol. Also, it produced highly significant elevation in the activity of CAT in kidney tissues of hyperlipidemic rats from 8.14 ± 1.53 u/gm in control hyperlipidemic to 22.38 ± 1.32 u/gm hyperlipidemic rats treated with eugenol as shown in table (2) and figure (3).

Administration of atorvastatin in hyperlipidemic rats produce no significant change in SOD activities in liver or kidney tissues from control hyperlipidemic rats as shown in table (2) and figure (2). However, its administration significantly increase the activity of CAT in liver tissues from 2.33 u/gm in control hyperlipidemic to 6.55 u/gm in atorvastatin treated group and highly significant increase the activity of CAT in kidney tissues from 8.14 u/gm in control hyperlipidemic to 19.8 u/gm in atorvastatin treated group (table 2 and figure 3).

Combined administration of both eugenol and atorvastatin resulted in highly significant increase the activity of SOD in liver tissues from 75.19 ± 8.87 u/gm in control hyperlipidemic rats to

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248.18±55.7u/gm in hyperlipidemic rats treated with both eugenol and atorvastatin. Also, there was highly significant increase in the activity of SOD in kidney tissues from 689.2±46.19 u/gm in control hyperlipidemic rats to 1438.9±397u/gm in hyperlipidemic rats treated by both eugenol and atorvastatin (table 2 and figure 2).

Eugenol and atorvastatin administration showed highly significant increase in the CAT activity of liver tissues from 2.33±1.06u/gm in control hyperlipidemic to 8.44±1.61u/gm in hyperlipidemic rats treated by both eugenol and atorvastatin. Also, there was highly significant increase the activity of CAT in kidney tissues from 8.14±1.53u/gm to 21.48±1.57 u/gm in hyperlipidemic treated with both eugenol and atorvastatin (table 2 and figure 3).

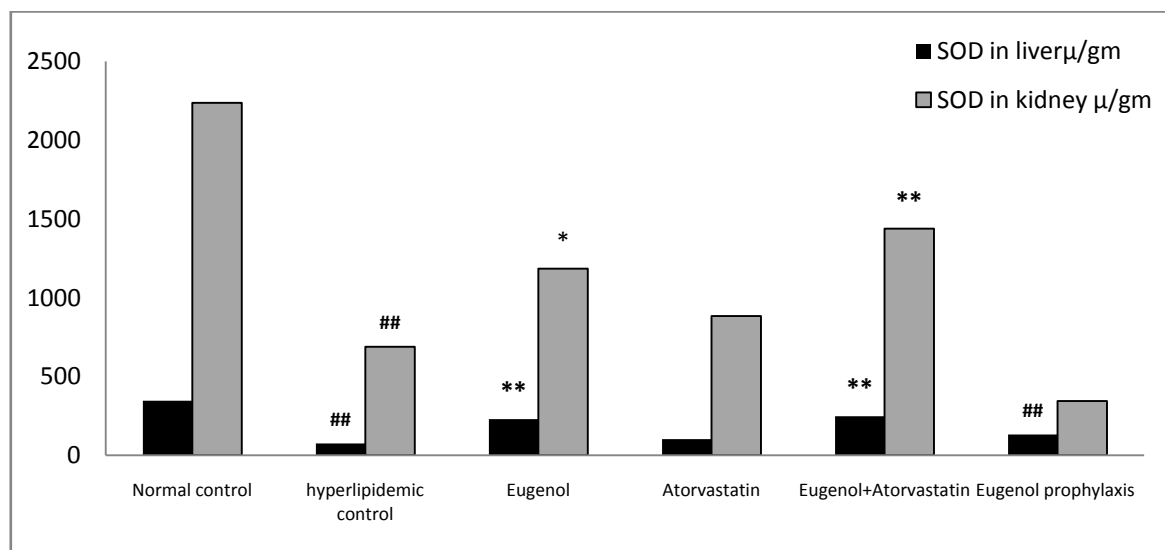
Administration of eugenol for 7days before induction of hyperlipidemia by triton produce no significant change in the activities of SOD in liver and kidney tissues and CAT in liver from control hyperlipidemic rats. But, there was highly significant increase in the activity of CAT in kidney tissues from 8.14±1.53 u/gm in triton induced hyperlipidemic rats or control hyperlipidemic to 13.96±1.29 u/gm (table 2, figures 2 and 3).

Table 2. Effect of eugenol (250 mg/kg), atorvastatin (40 mg/kg), eugenol+ atorvastatin and eugenol prophylaxis on SOD and CAT in liver and kidney tissues of triton induced hyperlipidemia in rats

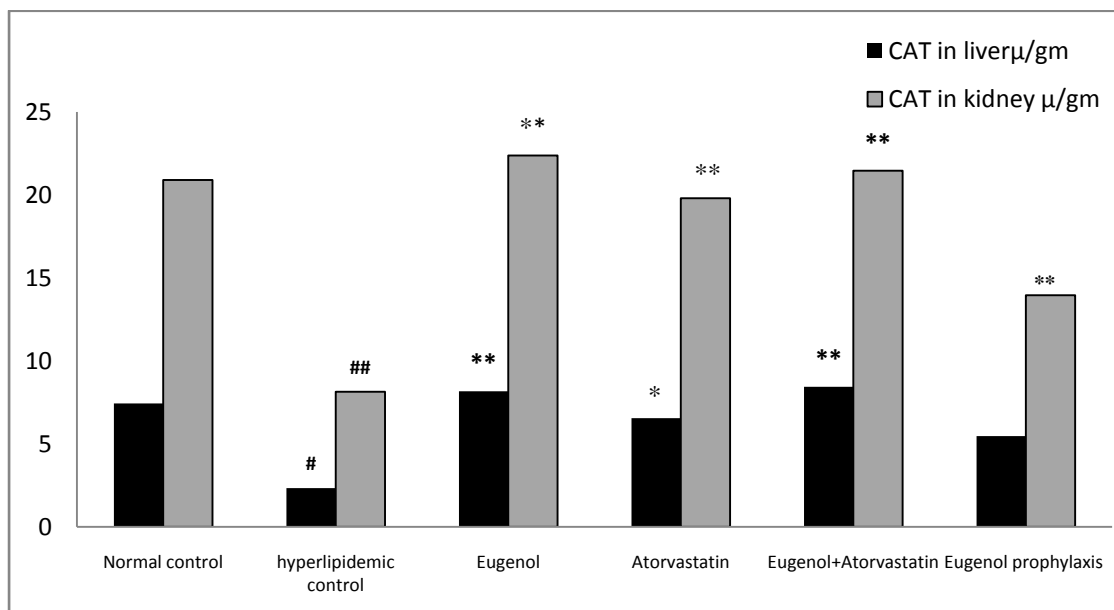
parameters	Normal control	Hyperlipidemic control	Hyperlipidemic +eugenol	Hyperlipidemic + atorvastatin	Hyperlipidemic + eugenol and atorvastatin	Hyperlipidemic pretreated with eugenol
SOD in liver (u/gm)	346±79.23	75.19 ^{##} ±8.87	229.16 ^{**} ±44.7	102.48±47.10	248.18 ^{**} ±55.7	138.42 ^{**} ±43.7
SOD in kidney (u/gm)	2236.4±308.7	689.2 ^{##} ±46.19	1184.2 [*] ±306	883.5±92.54	1438.9 ^{**} ±397	344.4±112
CAT in liver (u/gm)	7.44±3.55	2.33 [#] ±1.06	8.17 ^{**} ±2.44	6.55 [*] ±1.1	8.44 ^{**} ±1.61	5.47±1.16
CAT in kidney (u/gm)	20.9±2.58	8.14 ^{##} ±1.53	22.38 ^{**} ±1.32	19.8 ^{**} ±2.88	21.46 ^{**} ±1.75	13.96 ^{**} ±1.29

Data represent mean ± SD of 6 observations, [#] statistically significant from normal control $p < 0.05$, ^{##} statistically highly significant from normal control $p < 0.01$, ^{*} statistically significant from control hyperlipidemic $p < 0.05$, ^{**} statistically highly significant from control hyperlipidemic $p < 0.01$.

Fig 2. Effect of eugenol (250mg/kg), atorvastatin (40 mg/kg), eugenol and atorvastatin and eugenol prophylaxis on SOD of triton induced hyperlipidemia in rats.



Data represent mean ± SD of 6 observations, [#] statistically significant from normal control $p < 0.05$, ^{##} statistically highly significant from normal control $p < 0.01$, ^{*} statistically significant from control hyperlipidemic $p < 0.05$, ^{**} statistically highly significant from control hyperlipidemic $p < 0.01$.

Fig3. Effect of eugenol (250mg/kg), atorvastatin (40 mg/kg), eugenol and atorvastatin and eugenol prophylaxis on CAT of triton induced hyperlipidemia in rats

Data represent mean \pm SD of 6 observations, [#] statistically significant from normal control $p < 0.05$, ^{##} statistically highly significant from normal control $p < 0.01$, ^{*} statistically significant from control hyperlipidemic $p < 0.05$, ^{**} statistically highly significant from control hyperlipidemic $p < 0.01$.

5. DISCUSSION

Hyperlipidemia is a highly predictive risk factor for atherosclerosis, coronary artery diseases and cerebral vascular diseases. Coronary heart disease, stroke, atherosclerosis and hyperlipidemia are the primary causes of death (Pradeep et al., 2011).

Ischemic heart disease (IHD) is one of the leading causes of morbidity and mortality in developed and developing countries. The underlying mechanism of IHD involves the deposition and retention of serum lipids consisting of LDL cholesterol in the coronary arteries resulting in decreased blood flow to heart muscles. The treatment is based on the hypothesis that reduced cholesterol biosynthesis will decrease blood levels of cholesterol (Betsy et al., 2007).

Gotto (2006) reported that statins are inhibitors of 3-hydroxy-3-methylglutarylcoenzyme A reductase. Although, statins have been found to be effective in lowering the serum low-density lipid levels by as much as 21% to 43%, they have been found to cause many adverse effects. The association of hyperlipidemia with the development of atherosclerotic lesion has promoted widespread search for plant based compounds which safely and effectively control the lipid profile with least or no toxic effect (Zafar et al., 2010).

Clove is reported to possess aphrodisiac, antibacterial, antipyretic, anti-candidal, local anesthetic and antioxidant activities. The main component of clove bud oil is eugenol (80.3%), which is responsible for its odor, antiseptic and antioxidant properties (Anand et al., 2009).

Several studies have demonstrated the role of clove in reduction of total lipid profile and suggest its use as alternative and natural source for long term management of hyperlipidaemia (Shyamala et al., 2003, Jin et al., 2011 and Parle et al., 2011).

Cholesterol lowering effect of clove was reported by Parle and his college (2011) who showed that administration of clove powder with diet in three doses (400, 800, 1600 mg/kg) for 7 successive days reversed the memory deficits induced by ethanol and diazepam in mice. In addition, clove inhibited acetylcholine activity and lowered cholesterol level in mice and this was in agreement with our study.

Balasarikha et al., (2012) evaluated the effect of clove and turmeric in hyperlipidaemic patients. Clove supplemented group of hyperlipidaemic patients showed a maximum reduction in total cholesterol, triglycerides, LDL and VLDL cholesterol and an increase in the HDL cholesterol and this was in agreement with our results. The lipid lowering efficacy of clove may be due to inhibition of hepatic HMG-CoA reductase activity (El-Segaey et al., 2007).

Our results are in harmony with Jayaraman et al., (2013) as they investigated the effect of Citrulluscolocynthis in high fat diet induced hyperlipidaemia and streptozotocin induced diabetic rats, their results revealed that there were a significant reduction in CAT and SOD activities in high fat diet fed rats in comparison with control.

Interestingly, the results of our study showed that oral eugenol or atorvastatin significantly reverse the oxidative stress induced by hyperlipidemia, the concomitant administration of both eugenol with atorvastatin highly significant improves SOD and CAT activities.

Shyamala and his co-workers (2003) assessed the antioxidant efficacy and anti-hyperlipidemic effect of clove powder in rats fed with high fat diet; their results revealed that administration of clove increase the activities of SOD and CAT antioxidant enzymes in liver and kidney tissues. Also, there was significant reduction in high cholesterol and triglycerides level. Shyamala et al., (2003) concluded that co-administration of clove is suggested to have triggered the secretion of antioxidant enzymes in enhanced levels which in turn stopped the oxidative damage due to hyperlipidaemia. The anti-hyperlipidemic effect of clove may be due to its ability to combat oxidative stress by quenching free radicals generated in the body as a result of high fat diet. These results are in agreement with our results.

The antioxidant and anti-hyperlipidemic effects of eugenol were reported by Anbu et al., (2012) who studied the effect of eugenol on lipid levels, lipid peroxidation, enzymatic and non-enzymatic antioxidants in experimental hepatotoxic rats. Their results showed that oral administration of eugenol decreases the levels of cholesterol, free fatty acids, triglycerides and the extent of lipid peroxidation in ethanol exposed rats. Eugenol, a naturally occurring antioxidant, would scavenge the free radical produced by ethanol and this might be the reason for decreased lipid peroxidation products in rats treated with eugenol. Eugenol increased the activity of CAT and SOD in liver tissues and this is in agreement with the present study.

We assessed the protective role of eugenol before induction of hyperlipidemia, and the results showed highly significant reduction in total lipid profile and increased CAT activity in comparison with control hyperlipidemic but there was no significant difference in SOD activities. The protective effect of clove also was assessed by EL-Segaey et al., (2007), Eman et al., (2010) and Anbu et al., (2012).

These results suggest the use of moderate quantity of clove in diet as an antioxidant in offering protection against hyperlipidaemia. Also clove supplementation is economic and sustainable strategy in the management of hyperlipidaemia being a dietary intervention devoid of other possible side effects. Hence, eugenol may possibly be developed as an alternative cholesterol-lowering drug; however, further molecular studies are required to investigate the mechanism underlying the anti-hypercholesterolemic effect of this compound. Future studies must focus on the hypolipidemic effect of eugenol under conditions of chronic hypercholesterolemia.

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