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Research Article

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Cu $^{++}$ Induced *in Vitro* Antioxidant Impacts of *Allium sativum* on Low Density Lipoprotein (LDL) Oxidation in Type II Diabetic Patients

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ABSTRACT

Lipids when reacted with free radicals can undergo the highly damaging chain reaction of lipid peroxidation (LP) leading to both direct and indirect effects. Garlic (A*llium sativum*) is one of the most popular herbs used worldwide to reduce various risk factors associated with cardiovascular diseases. Result present in this study, demonstrate that diabetic patient experience on exaggerated oxidative stress when compared with Normal in addition, our result shows that in comparison to diabetic patient, modest and significant increase in plasma, glucose, TG,TC, VLDL-C, LDL-C, HDL-C, HDL2-C, HDL3-C and non-HDL-C levels in diabetic patient. This may be due to markedly increased production of oxidant and significantly diminished antioxidant defense including a decline in total plasma antioxidant. Based, *Allium sativum* mediated multiple therapeutic benefits described in the present study, daily intake of *Allium sativum* as dietary supplement by DM ,CVD, Atherosclerosis, CHD and hyperlepidemia may be useful in the prevention and treatment of DM include hyperlepidemia and atherosclerosis. In addition, daily intake of dietary *Allium sativum* will efficacious and cost effective.

Key words: Oxidized Cholesterol, Atherosclerosis, Hypolipidemic, Antioxidant, A*llium sativum*

INTRODUCTION

Diabetic mellitus is a chronic disorder resulting from a number of factors in which an absolute or relative deficiency of insulin or its function occurs. It is projected that by the year 2025, India alone would have 57 million diabetics mainly of type II disease constituting 90% of diabetic population [1].It is estimated that by the year of 2010 the total number of people with diabetes will reach 221 million, the area likely to be most affected being Asia and Africa [2]. The world wide incidence of type II diabetes is increasing and accounts for 6-12% of total expenditure in industrialized countries [3].Traditional Medicines derived from medicinal plants are used by about 60% of the world's population. Diabetes is an important human ailment afflicting many from various walks of life in different countries. In India it is proving to be a major health problem, especially in the urban areas. Though there are various approaches to reduce the ill effects of diabetes and its secondary complications, herbal formulations are preferred due to lesser side effects and low cost. A list of medicinal plants with proven antidiabetic and related beneficial effects and of herbal drugs used in treatment of diabetes is compiled. These include *Allium sativum, Eugenia jambolana, Momordica charantia Ocimum sanctum, Phyllanthus amarus, Pterocarpus marsupium, Tinospora cordifolia, Trigonella foenum graecum* and *Withania somnifera.* Garlic (A*llium sativum*) is one of the most popular herbs used worldwide to reduce various risk factors associated with cardiovascular diseases. Garlic, a member of the *Liliaceae* family, is a common food for flavour and spice and it is one of the herbs most commonly used in modern folkloric medicine [4].

FIGURE 1: A*llium sativum* Clove **FIGURE 2: A*llium sativum* Plant**

Membrane lipids present in subcellular organelles are highly susceptible to free radical damage. Lipids when reacted with free radicals can undergo the highly damaging chain reaction of lipid peroxidation (LP) leading to both direct and indirect effects. During LP a large number of toxic byproducts are also formed that can have effects at a site away from the area of generation, behaving as 'second messengers'. The damage caused by LP is highly detrimental to the functioning of the cell. Lipid peroxidation is a free radical mediated process [5].LDL oxidation is generally believed to occur mainly in the intima of the artery in microdomains sequestered from antioxidants. Several lines of evidence observed by different groups over the years support a role for Ox-LDL in atherogenesis [6]. LDL can be oxidatively modified in a cell-free system by transition metals such as iron and copper and by all the major cells of the arterial wall such as endothelial cells, smooth muscle cells, and monocyte-macrophages. Physiologically relevant mechanisms underlying LDL oxidation *in vivo* are yet to be established. Various studies implicate superoxide anion as one agent that promotes oxidation of LDL lipids, mediated by smooth muscle cells and phagocytes. In this we investigated the efficacy of antioxidant and hypolipidemic agent *Allium sativum* by analyzing all the parameters in plasma lipoprotein lipids, TL, TC, TG, VLDL-C, and LDL-C, non-HDL-C, MDA and *in-vitro* oxidizability of LDL, with and without A*llium sativum*.

MATERIAL AND METHOD

SUBJECT AND STUDY DESIGN

The study protocol was approved by the Department of Biomedical Science in Dolphin (P.G.) Institute Of Bio-Medical & Natural Sciences Dehradun. Diabetic mellitus type II patients and normal control subject were recruited from Doon & GTB Diabetics research centre Dehradun. Informed consent from study enrollment was obtained from each of the study subject. All other chemicals and reagents used in this study were of analytical grade.

PLANT MATERIAL

Collection of plant parts-The bulbs of garlic (*Allium sativum*) were collected from localities Of Dehradun, Uttrakhand and evaluated for their antioxidant & antimicrobial activity.

Preparation of Aqueous and Ethanolic Garlic Extract- 50g of dried plant sample was powdered into course size which was then used for extraction by soxhlet extractor using ethanol (300ml) for 24 hours. The extract was then filtered and concentrated in a rotavapor and dried under vacuum at 600C. The marc left out was extracted with water by refluxing for 4hrs and dried extract was prepared as per the earlier procedure.

ANALYTICAL PROCEDURES

Collection of blood-The blood samples of diabetic mellitus and normal persons were collected from Doon hospital & Guru Teg Bahadur Diabetic Research Centre, Dehradun. Plasma was separated from blood by centrifugation at 2,500 rpm for 30 min, aliquoted and either stored at 40C or frozen at –200C for use in other experiments.

Determination of parameters-Determination of Triglycerides [7], LDL isolation [8], isolation of high density lipoprotein (HDL), HDL2 and HDL3 subtractions [9], *in vivo* and *in vitro* Cu++-mediated oxidation of LDL [10,11], total antioxidant power by FRAP assay [12].

RESULTS

1. AVERAGE VALUE OF AGE, WEIGHT, MALE, FEMALE, DIABETIC HISTORY (D/HISTORY) AND TREATMENT OF NORMAL AND DIABETIC MELLITUS TYPE II PATIENTS.

Figure 3, indicated that the average value of age, weight, male, female, D/history of patient and their treatment of DM type II and normal control subjects. No significant changes in these parameters were observed when compared to their corresponding values of normal subjects.

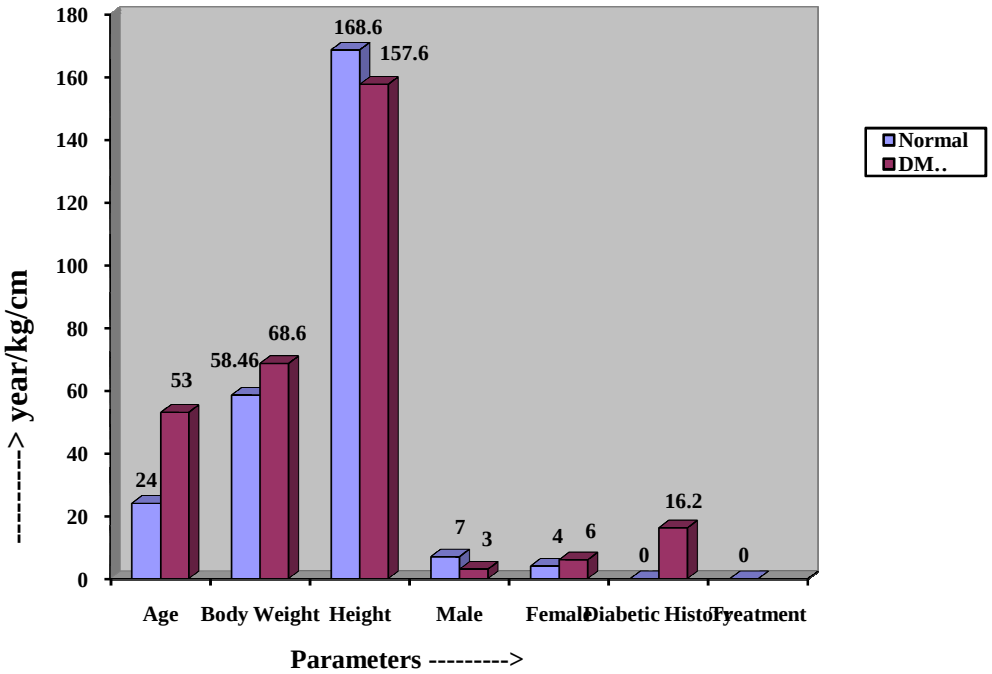


FIGURE 3: Average Value of Different Parametrs of Normal and Diabetic Mellitus Type II Patients.

1. **AVERAGE VALUES OF GLUCOSE AN TRIGLYSRIDES (TGs) OF NORMAL CONTROL AND DIABETIC MELLITUS (DM)TYPE –II.**

The value of Glucose and TGs significantly increased from normal control value 101.82 ±1.76 mg /dl ,100.22 ± 2.82 mg /dl respectively of normal control to 258.6 ± 1.42 mg /dl (+153.97%), 254.18 ± 2.24 mg /dl (+ 153.62%) in D/M Type-II as shown in Figure 4. This result demonstrate that significantly increase in the level of Glucose and TGs is due to oxidative stress which may increase the cardiovascular diseases (CVD) in the population.

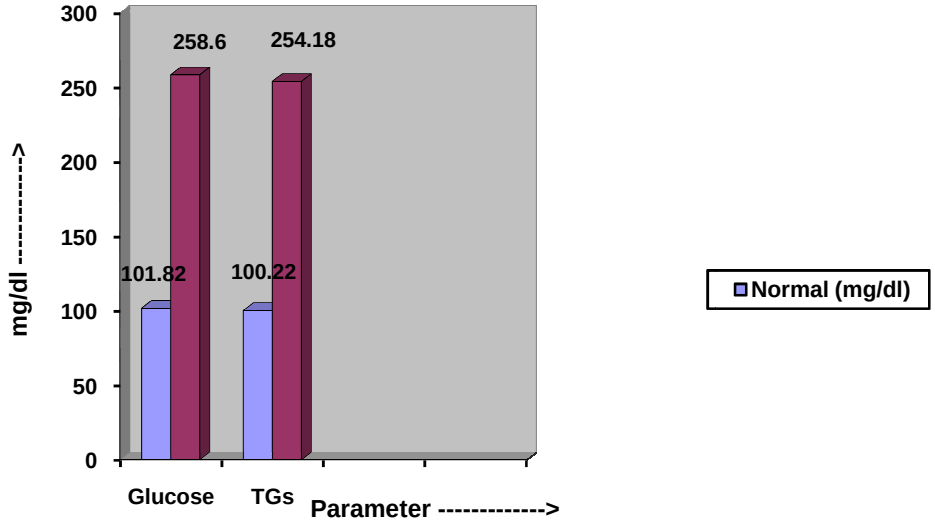
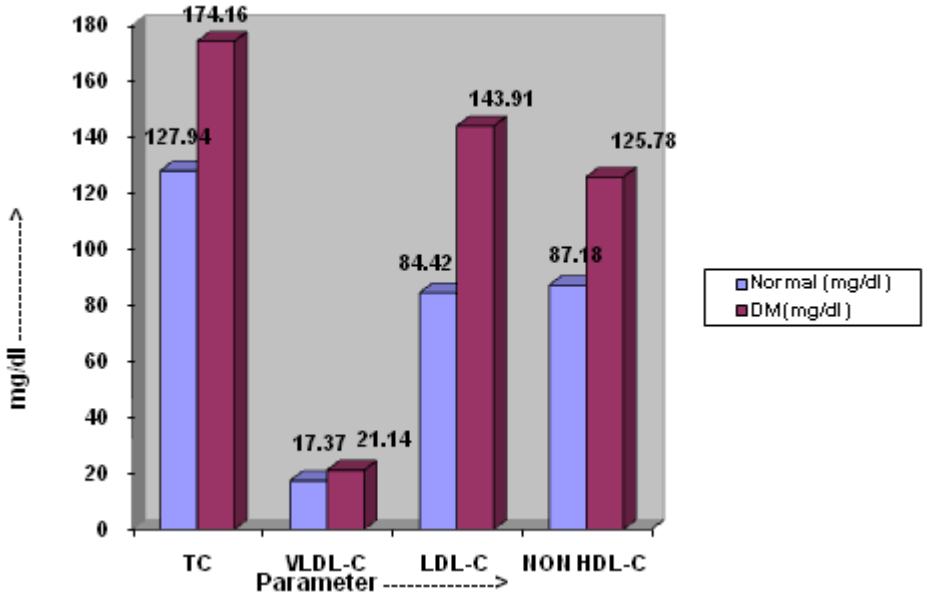


FIGURE 4: Average Value of Glucose and TG of Normal and Diabetic Mellitus Type II Patients.

2. **AVERAGE VALUE OF TOTAL CHOLESTEROL (TC), VERY LOW DENSITY LIPOPROTEIN (VLDL), LOW DENSITY LIPOPROTEIN (LDL), NON HIGH DENSITY LIPOPROTEIN (NON HDL) OF NORMAL AND DIABETIC MELLITUS TYPE II.**



Values of TC, VLDL-C , LDL-C, Non HDL-C significantly increase from normal control value 127.94 ± 1.84 mg/dl, 17.37 ± 0.42 mg/dl, 84.42 ± 1.73mg/dl and 87.18 ± 0.98 mg/dl respectively of normal control to 174.16 ± 2.62 mg/dl (+36.12%), 21.14 ± 0.37 mg/dl(+21.70%), 143.91 ± 1.72 mg/dl (+ 70.46%) and 125.78 ± 0.90 mg/dl (+ 44.27%) of D/M Type-II. This result demonstrates that significantly increase in the level of TC, VLDL-C, LDL-C, Non HDL-C is due to oxidative stress which may increase the CVD in the population.

FIGURE 5: Average Value Of Total Cholesterol (TC), Very Low Density Lipoprotein (VLDL), Low Density Lipoprotein (LDL), Non High Density Lipoprotein (NON HDL) Of Normal and Diabetic Mellitus Type II

5: AVERAGE VALUE OF HIGH DENSITY LIPOPROTEIN (HDL-C) AND ITS SUBFRACTIONS (HDL2-C AND HDL3-C) IN NORMAL AND DIABETIC MELLITUS TYPE II.

As shown in Figure 6, the values of HDL-C, HDL2-C, HDL3-C significantly increase from normal control value 39.92±1.37, 14.93±0.58, 28.92±0.82respectively of normal control to 51.87±1.76 (+29.93%), 17.23±0.68 (+15.40%), 35.58±1.38 (+23.02%)respectively in DM type II.Our result demonstrates that significantly increase in the level of HDL-C, HDL2-C, HDL3-C is due to the oxidative stress which may increase the CVD in the population.

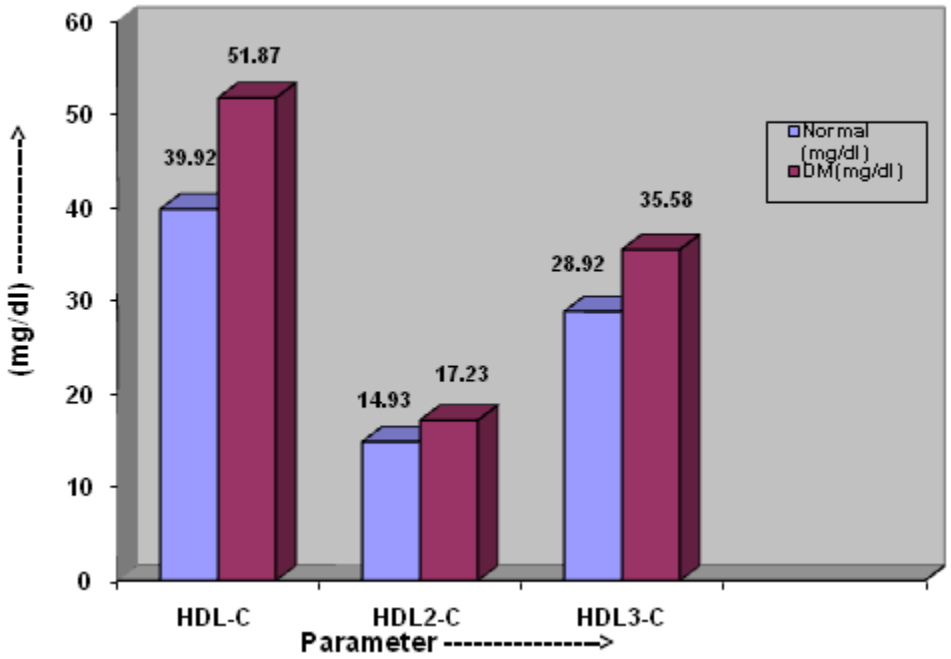


FIGURE 6: Average Value Of High Density Lipoprotein (HDL-C) And Its Subfractions (HDL2-C AND HDL3-C) In Normal And Diabetic Mellitus Type II.

6. MEASUREMENT OF TOTAL ANTIOXIDENT POWER IN *Allium sativum* (GARLIC) AT DIFFERENT CONCENTRATIONS.

The impact of total antioxidant power of *Allium sativum* (garlic) at different concentrations 10 µl/mg, 20 µl/mg, 50 µl/mg, 60 µl/mg, 90 µl/mg ,100 µl/mg significantly increase from 1.318 n mole/mg, 1.5627 n mole/mg(+24.47%),1.7245 n mole/mg(+40.65%), 1.8352 n mole/mg(+51.72%), 1.9289 n mole/mg(+6109%), 1.9368 n mole/mg(+61.88%), when compared to basal value 10 µl/mg (1.381 n mole/mg.

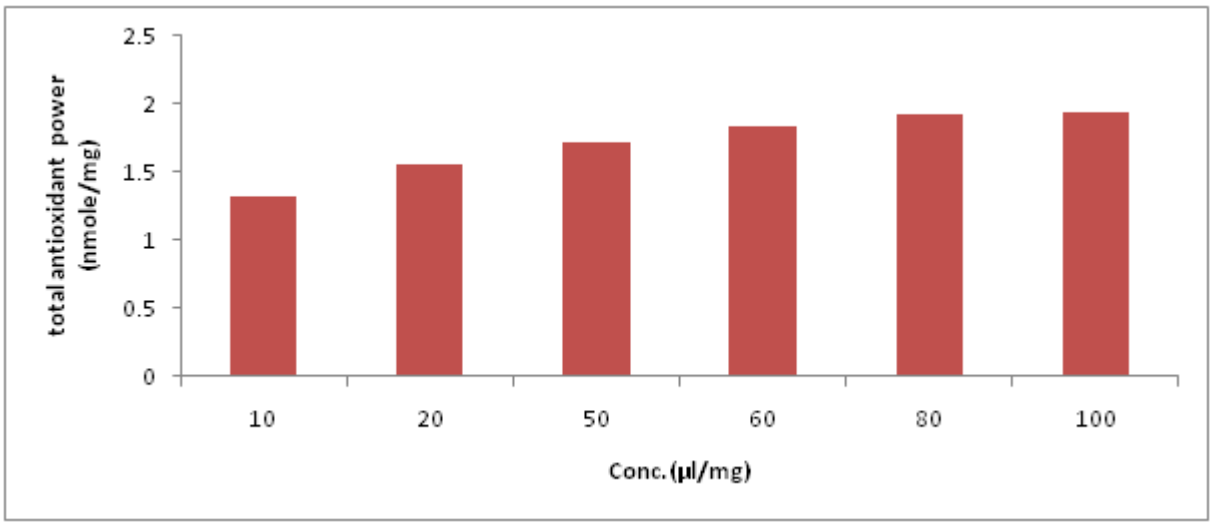


FIGURE 7: Total Antioxidant Power in *Allium sativum* (GARLIC)

7. IN VITRO ANTIOXIDENT IMPACT OF BASAL VALUE OF CONJUGATED DIENE FORMATION IN LDL ISOLATED FROM NORMAL AND D/M TYPE II.

The *ex-vivo* baseline diene conjugation (BDC) levels of LDL, in DM Type-II was increased by (+26.46%), in comparison to the corresponding normal control value. After the initiation Cu++ mediated oxidation of LDL in each group were significantly increased (+57.44%) and (+46.19%) in normal and DM type II respectively. As expected in vitro LDL oxidation was carried out in the presence of *Allium sativum* (garlic) (10µl). as shown in Table 1 in normal control and DM type II, addition of *Allium sativum* (garlic) (10µl) during LDL oxidation decreased the maximal amount of conjugated diene by (-29.47%) and (-28.92%), respectively as compared to maximal conjugated diene values of LDL oxidation after 4 hrs incubation. These results indicate a strong antioxidative production of LDL by *Allium sativum* (garlic) in D.M. Type-II.

Table 1: LDL oxidation with or without *Allium sativum* (garlic)

Conc.(µl)	Incubation Time at 370C	Conjugated Diene formation (nmoles/mg protein)	
		Normal	DM
10	Zero minute	176.39±0,048*	229.26±0.032*
10	CuSo4 (4 hrs)	286.33±0.023*	335.16±0.032*
10	CuSo4 + garlic (4 hrs)	201.92±0.12*	238.18±0.048*

DISCUSSION

Results from the present study, indicated that the average value of age, weight, male, female, D/history of patient DM type II and normal control subjects. No significant changes in these parameters were observed when compared to their corresponding values of normal subjects. The value of Glucose and TGs significantly increased from normal control value 101.82 ±1.76 mg /dl ,100.22 ± 2.82 mg /dl respectively of normal control to 258.6 ± 1.42 mg /dl (+153.97%), 254.18 ± 2.24 mg /dl (+ 153.62%) in D/M Type-II as shown in Figure 4. This result demonstrate that significantly increase in the level of Glucose and TGs is due to oxidative stress which may increase the cardiovascular diseases (CVD) in the population. On the othe hand, Values of TC, VLDL-C , LDL-C, Non HDL-C significantly increase from normal control value 127.94 ± 1.84 mg/dl, 17.37 ± 0.42 mg/dl, 84.42 ± 1.73mg/dl and 87.18 ± 0.98 mg/dl respectively of normal control to 174.16 ± 2.62 mg/dl (+36.12%), 21.14 ± 0.37 mg/dl(+21.70%), 143.91 ± 1.72 mg/dl (+ 70.46%) and 125.78 ± 0.90 mg/dl (+44.27%) of D/M Type-II. This result demonstrates

that significantly increase in the level of TC, VLDL-C, LDL-C, Non HDL-C is due to oxidative stress which may increase the CVD in the population. As shown in Figure 6, the values of HDL-C, HDL2-C, HDL3-C significantly increase from normal control value 39.92±1.37, 14.93±0.58, 28.92±0.82respectively of normal control to 51.87±1.76 (+29.93%), 17.23±0.68 (+15.40%), 35.58±1.38 (+23.02%)respectively in DM type II.Our result demonstrates that significantly increase in the level of HDL-C, HDL2-C, HDL3-C is due to the oxidative stress which may increase the CVD in the population. The impact of total antioxidant power of *Allium sativum* (garlic) at different concentrations 10 µl/mg, 20 µl/mg, 50. µl/mg, 60 µl/mg, 90 µl/mg ,100 µl/mg significantly increase from 1.318 n mole/mg, 1.5627 nmole/mg(+24.47%),1.7245 n mole/mg(+40.65%), 1.8352 n mole/mg(+51.72%), 1.9289 n mole/mg(+6109%), 1.9368 n mole/mg(+61.88%), when compared to basal value 10 µl/mg (1.381 n mole/mg. The *ex-vivo* baseline diene conjugation (BDC) levels of LDL, in DM Type-II was increased by (+26.46%), in comparison to the corresponding normal control value. After the initiation Cu++ mediated oxidation of LDL in each group were significantly increased (+57.44%) and (+46.19%) in normal and DM type II respectively. As expected in vitro LDL oxidation was carried out in the presence of *Allium sativum* (garlic) (10µl). as shown in Table 1 in normal control and DM type II, addition of *Allium sativum* (garlic) (10µl) during LDL oxidation decreased the maximal amount of conjugated diene by (-29.47%) and (-28.92%), respectively as compared to maximal conjugated diene values of LDL oxidation after 4 hrs incubation. These results indicate a strong antioxidative production of LDL by *Allium sativum* (garlic) in D.M. Type-II. Therefore, *Allium sativum* may exert their cholesterol lowering effect in hyperlipidemic rats in a similar manner as previously reported for hyperlipidemic animals [5, 6] and humans [5, 13]. Mechanism wise, as previously shown in HepG2 cells, as well as in normolipidemic and hyperlipidemic rats, tocotrienols reduce cholesterol synthesis by suppressing HMG-CoA reductase activity, which in turn is reduced by a decline in its protein mass [6, 14]. The decline in protein mass may be achieved by inhibition of HMG-CoA reductase synthesis and/orenhanced degradation. Consistent with in vivo results in rats 6, γ-tocotrienol has been shown to mediate the suppression of enzymatic activity and protein mass of HMG-CoA reductase in HepG2 cells through decreased synthesis (57 % of control) and enhanced degradation (2.4-fold versus control) of the enzyme [14]. Based, *Allium sativum* mediated multiple therapeutic benefits described in the present study, daily intake of *Allium sativum* as dietary supplement by DM ,CVD, Atherosclerosis, CHD and hyperlepidemia may be useful in the prevention and treatment of DM include hyperlepidemia and atherosclerosis. In addition, daily intake of dietary *Allium sativum* will efficacious and cost effective.

CONCLUSION

Diabetes mellitus (DM) is a metabolic disorder of the endocrine system. The disease occurs worldwide and its incidence is increasing rapidly in most parts of the world. People suffering from diabetes are not able to produce or properly use insulin in the body, so they have a high level of blood glucose. DM is a metabolic disorder affecting carbohydrate, fat and protein metabolism. Result present in this study demonstrate that diabetic patient experience on exaggerated oxidative stress when compared with Normal in addition, our result shows that in comparison to diabetic patient, modest and significant increase in plasma, glucose, TG,TC, VLDL-C, LDL-C, HDL-C, HDL2-C, HDL3-C and non-HDL-C levels in diabetic patient. This may be due to markedly increased production of oxidant and significantly diminished antioxidant defense including a decline in total plasma antioxidant.

Based, *Allium sativum* mediated multiple therapeutic benefits described in the present study, daily intake of *Allium sativum* as dietary supplement by DM ,CVD, Atherosclerosis, CHD and hyperlepidemia may be useful in the prevention and treatment of DM include hyperlepidemia and atherosclerosis. In addition, daily intake of dietary *Allium sativum* will efficacious and cost effective.

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