

**EFFECT OF *AMMOMUM SUBULATUM* ON OXIDATIVE STRESS
AND ATHEROSCLEROSIS IN CHOLESTEROL FED RABBITS**

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Summary

We have studied the effects of *Ammomum subulatum* and its antioxidant activity in cholesterol fed rabbits. The results confirmed that the total and LDL-cholesterol, phospholipid, triglyceride and VLDL-cholesterol levels were significantly increased in serum and aortic tissue of rabbits fed with a high cholesterol diet (500 mg/kg.b.wt./day) for 90 days. The HDL-cholesterol/Total cholesterol ratio was reduced significantly after cholesterol feeding. A significant increase in aortic lipid peroxidation (high MDA level) and reduction in glutathione (GSH) and catalase activity was observed in cholesterol fed rabbits. Oral administration of 70% methanolic extract of *A. subulatum* seeds at the doses 150 and 250 mg/kg b.wt./day showed a significant reduction in the serum and tissue content of total cholesterol, phospholipids and triglycerides. The serum HDL-cholesterol/ Total cholesterol ratio was raised whereas LDL and VLDL-cholesterol levels were reduced significantly after treatment. The seeds of *A. subulatum* possessed antioxidant activity as shown by increased GSH and catalase activities and decreased malanodialdehyde (MDA) levels. Histopathological studies showed a well developed atheromatous plaques throughout the aorta after cholesterol feeding in comparison to control rabbits. Treatment with *A. subulatum* exhibited a significant regression in plaque size of aorta. These findings suggest that orally administered *A. subulatum* could be useful in prevention of hyperlipidaemia and provide antioxidant protection.

Key words: *A. subulatum*, HDL and LDL cholesterol, hyperlipidaemia, antioxidant enzymes, rabbits.

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Coronary heart disease is the cause of more than half of all cardiovascular disease mortality (1). Hyperlipidaemia is one of the major contribution to the atherosclerosis and CHD in our society (2). Oxidation of low density lipoprotein cholesterol (LDL-C) is a strong predictor of coronary heart disease (CHD) that initiate the endothelial dysfunction which promotes a series of stages beginning with fatty streak lesions composed largely of lipid engorged macrophage foam cells and ultimately progressing to complex plaques. These plaques provide a barrier to arterial blood flow and encroaches on the lumen (3).

Reduction of serum cholesterol concentration is one of the aim in prevention of the CHD (4) and experimental studies in different animal models showed that antioxidants decreases oxidation of low-density lipoprotein cholesterol and reduces plaque formation (5,6).

Researchers have began to formally study the health benefits of herbs and spices that have been used for thousand years to treat illness and flavour foods (7). These spices and herbs contain potent antioxidant compound that provides significant protection against chronic diseases and protect LDL-cholesterol from oxidation, which is thought to play a key role in pathogenesis of atherosclerosis (8,9).

Ammomum subulatum (Greater cardamom) is one of the world's very ancient spices and has also been universally used throughout history for its health benefits. It is native to the east originating in the forests of the Western Ghats in southern India. Today it also grows in Srilanka, Guatemala, Indochina and Tanzania. The seeds contain the glycosides, petunidine 3,5-diglucoside and leucocyanidin-3-O- β -D-glucopyranoside, and a new aurone glycoside subulin. A subulatum extract possesses antioxidative characteristics since it can scavenge free radicals such as O_2^- and H_2O_2 (10, 11).

In the present study we investigated the effect of *A. subulatum* extract on the development of atherosclerosis in relation to plasma cholesterol levels and the resistance of LDL to atherogenic modification such as oxidation.

Material and Method

Test material: *A. subulatum* belongs to family Zingiberaceae and is commonly known as Greater Cardamom or Doda. Authentic seeds of *A. subulatum* obtain from national Institute of Ayurveda, Jaipur, was powdered and extracted with 70% methanol for 36-48 hrs by soxhlet extraction method. then methanol was separated under reduced pressure to obtain solid mass.

Animal model: Twenty adult rabbits weighing 1.5 - 2.0 Kg were used. Rabbits were housed in animal cages at constant temperature and maintained on a standard pellet diet (Ashirwad Industries, Chandigarh), green leafy vegetables and water *ad libitum*.

Experimental design : The rabbits were divided into the following group of four animals each.

Group A: Served as (vehicle only) control.

Group B: Received 500mg cholesterol/kgb.wt./day in 5 ml coconut oil for 90 days.

GroupC: Received 500mg cholesterol/kg b.wt./day in 5ml coconut oil + *A. subulatum* seeds extract (150 mg/kg b.wt./day) for 90 days.

Group D: Received 500 mg cholesterol / Kg b.wt. /day in 5ml coconut oil + *A. subulatum* seeds extract (250 mg/kg b.wt. / day) for 90 days.

At the end of the experiment all the rabbits were sacrificed. The blood was collected through cardiac puncture. Serum was separated by centrifugation and stored at -20°C until analysis. Aorta was removed quickly, cleared off fat and connective tissue and prepared for histopathological examination.

Parameters studied : Following biochemical parameters have been investigated in serum and tissue after treatment with *A. subulatum* seed extract i.e. Total cholesterol (12), Phospholipid (13), Triglyceride (14), HDL cholesterol (15), LDL and VLDL-Cholesterol (16), Lipid peroxidation (17), glutathione (18) and catalase (19).

Statistical analysis : Statistical analysis of data were done using student's 't' test, and results were expressed as means \pm SD. the values at ($P\leq 0.01$; $P\leq 0.001$) were considered significant.

Results

Serum biochemistry (Fig. 1 and 2) : The results presented in fig. 1 indicated that the cholesterol fed rabbits showed a significant elevation ($P\leq 0.001$) in serum total cholesterol, triglyceride and phospholipid levels as compared to normal group. A significant increase ($P\leq 0.001$) in LDL and VLDL cholesterol was noticed after cholesterol feeding. The serum HDL-C/Total cholesterol ratio was reduced significantly ($P\leq 0.001$) after cholesterol feeding. Oral administration of *A. subulatum* extract along with cholesterol feeding caused a significant reduction in serum total cholesterol, triglyceride and phospholipid and LDL cholesterol levels of rabbits. VLDL cholesterol level showed non-significant change after treatment with low dose level of *A. subulatum* whereas high dose level showed a significant reduction ($P\leq 0.01$). A non-significant change was observed in HDL-C/Total cholesterol ratio after treatment with *A. subulatum*. The higher dose level i.e. 250mg showed significant reduction ($P\leq 0.01$) in this ratio.

Tissue biochemistry (Fig. 3) : Cholesterol feeding to rabbits caused a significant increase ($P\leq 0.001$) in total cholesterol, triglyceride and phospholipid contents of aorta in comparison to control. Simultaneous treatment of *A. subulatum* and cholesterol exhibited a significant decline ($P< 0.01$, $P< 0.001$) in the levels of total cholesterol, triglyceride and phospholipid of aorta at both the dose levels, respectively.

Antioxidant parameters (Fig. 4, 5 and 6) : Oral administration of cholesterol alone in rabbits exhibits a significant increase ($P< 0.001$) in aortic lipid peroxidation when compared with normal group of rabbits whereas significant reduction ($P\leq 0.001$) was observed in the catalase and glutathione (GSH) activity. A dose dependent elevation was observed in the catalase and GSH activity after treatment with *A. subulatum* and cholesterol. A significant fall ($P\leq 0.01$) in aortic lipid peroxidation was also noticed after treatment with *A. subulatum* at both the doses.

Histopathological findings : The control rabbit aorta (Fig. 7A) showed all the three layers i.e. tunica intima, tunica media and the outer most tunica adventitia. The aorta of atherodiet treated rabbits (Fig. 7B) showed the large atheromatous plaque by cell proliferation. Marked histological changes were observed in the aorta of *A. subulatum* treated rabbits in a dose dependent manner (Fig. 7C,7D). Aortic plaque was almost completely regressed and lumen size was also restored near to normal.

Discussion

Dietary factors play a key role in the development of various diseases including cardiovascular disease. Epidemiological studies have shown that diet rich in fruits, herbs and spices are associated with low risk of cardiovascular disease (20). In the present study the hypolipidaemia and antioxidant effects of cardamom (*A. subulatum*) were evaluated in rabbits fed with high fat diet.

Cholesterol feeding to rabbits resulted a significant increase in serum total cholesterol, triglycerides, phospholipids, LDL and VLDL-cholesterol levels (21,22). Our study demonstrated that oral administration of *A. subulatum* reduced the serum total cholesterol in a dose dependent manner. The lowering of serum cholesterol is believed to be largely due to a reduction in LDL-cholesterol (23), which may be due to an inhibition of hepatic cholesterol biosynthesis possibly via reduction in the activity of 3HMG-CoA reductase, an enzyme that catalyses the rate limiting steps of cholesterol synthesis (24) thereby causing increased expression functional LDL receptors on cells which in turn lowers serum cholesterol (25, 26). A meta-analysis of several prospective studies confirmed that increased level of plasma cholesterol are transported from peripheral cells to the liver by reverse cholesterol transport, a pathway that may protect against atherosclerosis by clearing excess cholesterol from arterial wall (27).

Further, reduction in total cholesterol, triglyceride and phospholipid contents of aortic tissue may be suggestive of a beneficial role of the drug, thereby showing anti-atherosclerotic potential of the plant extract (28,29).

The antiatherogenicity of *A. subulatum* extract could also be attributed to its direct antioxidative effects. It has the ability to activate antioxidant enzymes that can help prevent certain diseases such as atherosclerosis which can be brought on by free radical oxidizing low density lipoprotein cholesterol, protect against endothelial dysfunction, further prevents the formation of aortic atheroma (30).

In the present study, significant increase of TBARS (Measurement of lipid peroxidation) activity in aorta was observed in cholesterol fed rabbits (31). The increase level of TBARS in the aortic wall is a well known index of lipid peroxidation, emphasize the occurrence of vascular oxidative stress. Our findings showed that administration of *A. subulatum* extract along with cholesterol caused significant reduction in the level of TBARS in aorta indicating antilipid peroxidative nature of plant extract (32).

Glutathione (GSH) is a tripeptide of amino acids and is essential to maintain structural and functional integrity of the cells, is a major free radical scavenger was increased after treatment with *A. subulatum* by showing protection from oxidative stress induced by cholesterol feeding (33). Similarly a significant increase in catalase activity after simultaneous administration of *A. subulatum* was observed in aorta of rabbits as compared to hypercholesterolemic rabbits.

Triglyceride is a strong predictor of cardiovascular disease (34). Cholesterol feeding elevates the concentration of serum triglyceride which can be due to reduction in lipoprotein lipase (LPL) activity, an enzyme which is involved in the uptake of triglyceride rich lipoproteins by extra hepatic tissue (35). Lipoprotein lipase mediates the hydrolysis of triglycerides of chylomicrons and VLDL to generate free fatty acids and glycerol. Reduction in serum triglycerides concentration after administration of *A. subulatum* extract at the different doses may be due to either decrease in VLDL synthesis or increase in LPL activity, which results in lowering of triglycerides (36).

Maximum reduction in triglyceride levels was observed at the dose level of 250mg/kg.b.wt./day. Administration of cholesterol caused a significant increase in the level of serum phospholipid as compared to normal group of rabbits. These elevated levels were brought near to normal values after treatment with *A. subulatum*. It has been suggested that an elevation or reduction in serum phospholipids might be due to any disturbance in the anabolism or catabolism of very low density lipoprotein (VLDL) cholesterol (37).

Dietary cholesterol causes significant elevation in LDL as well as VLDL cholesterol. The mechanism behind this is that dietary cholesterol probably suppress the synthesis of LDL receptors (38) when cellular cholesterol falls, the synthesis of new receptors increases. The decrease in serum LDL and VLDL cholesterol after treatment with *A. subulatum* may be due to an increased uptake by extrahepatic tissues, particularly of VLDL as it is believed to be the precursor of LDL (39). Consumption of *A. subulatum* extract increases the HDL ratio when compared to cholesterol fed rabbits. It has been suggested that HDL functions to transport cholesterol from peripheral tissue to the liver.

Histopathological studies of aorta revealed a large amount of atheromatous plaque in arterial wall after cholesterol feeding. Initiation of plaque followed by the oxidation of low density lipoprotein cholesterol which promotes localized endothelial damage and increases penetration of LDL into the intima (40). Reduction in the plaque area after treatment with *A. subulatum* may be due to lowering serum cholesterol and oxidative stress in aorta and significant improvement in antioxidant enzymes (41).

In conclusion, administration of *A. subulatum* extract could be more beneficial in preventing hyperlipidaemia. It has the ability to activate antioxidant enzymes that reduces peroxidation in aortic tissues. There was also a reduction in atherosclerotic plaque area. The protective activity of *A. subulatum* extract may be due to its antioxidant defense system and that reduction of LDL oxidation may provide a protective effect against the detrimental action of oxidized LDL.

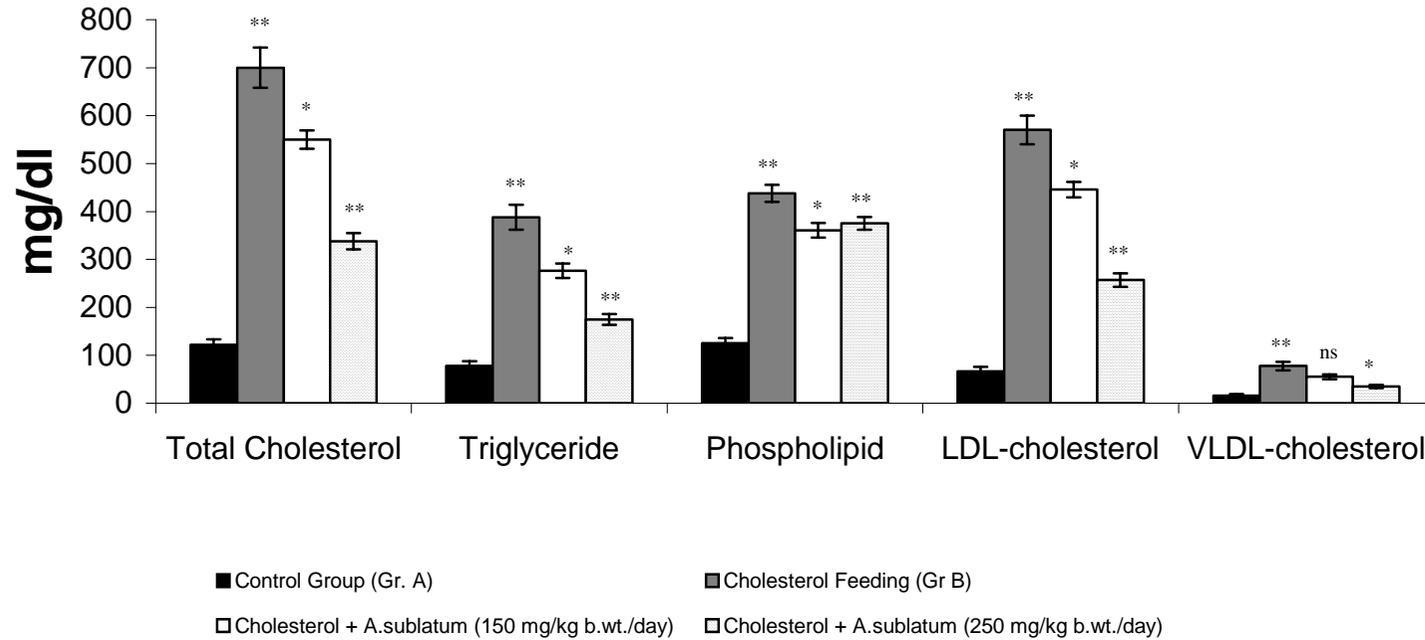
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Fig. 1 : Effects of *A. subulatum* on Serum Lipid Profile



* $P \leq 0.01$ Significant

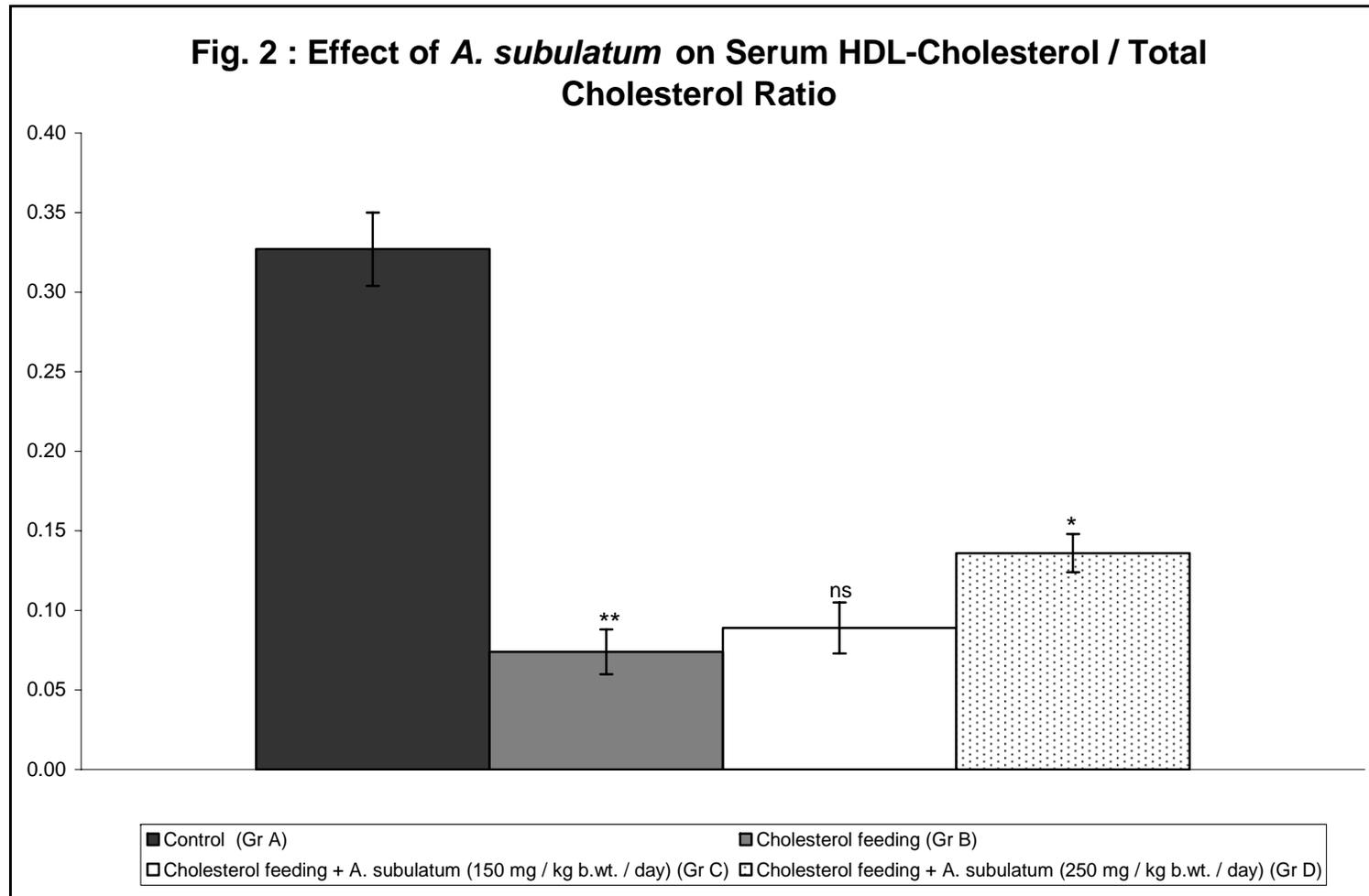
** $P \leq 0.001$ Highly Significant

Group B

ns = Non Significant

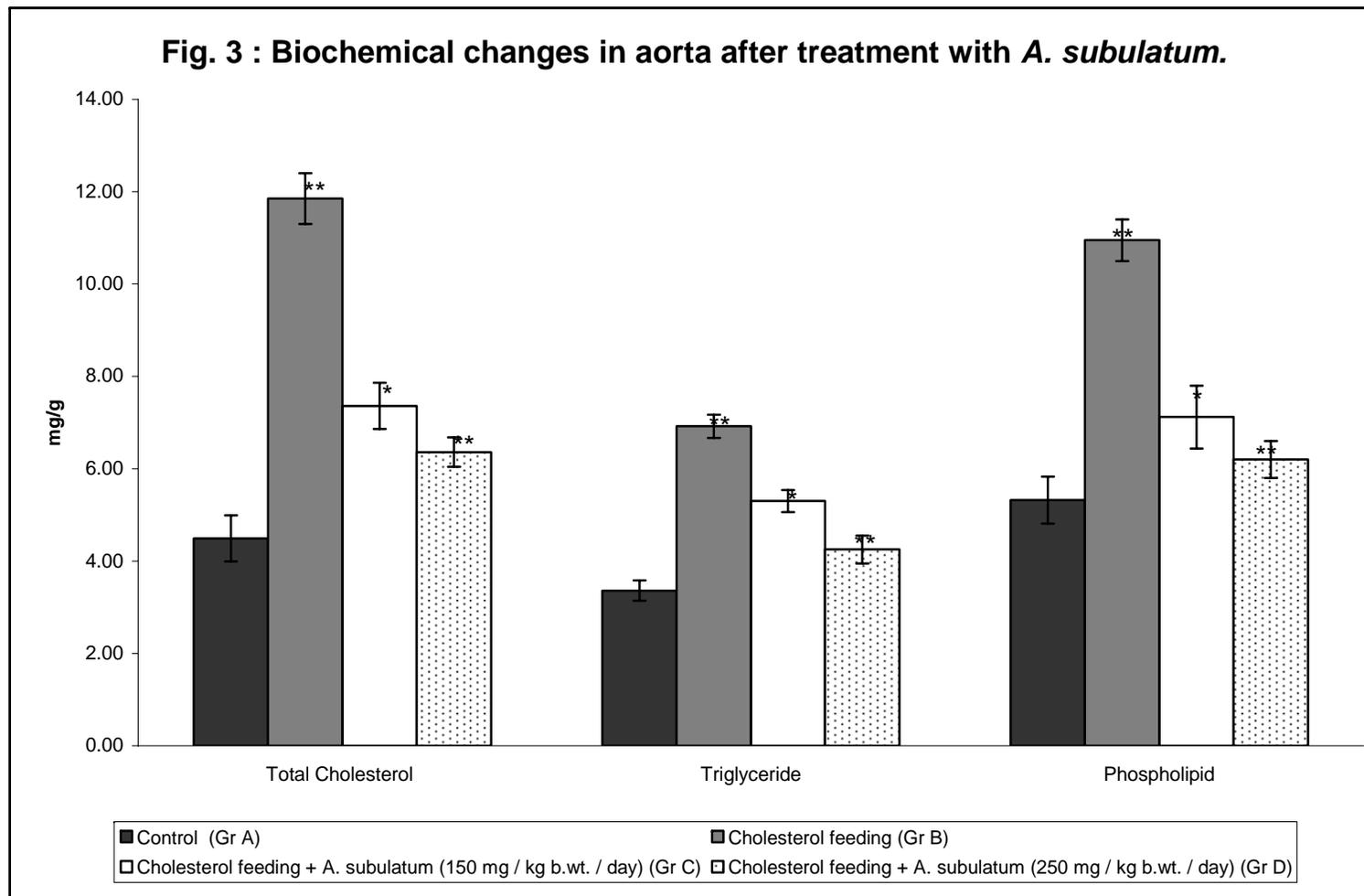
Group B Compared with Group A

Group C and D Compared with



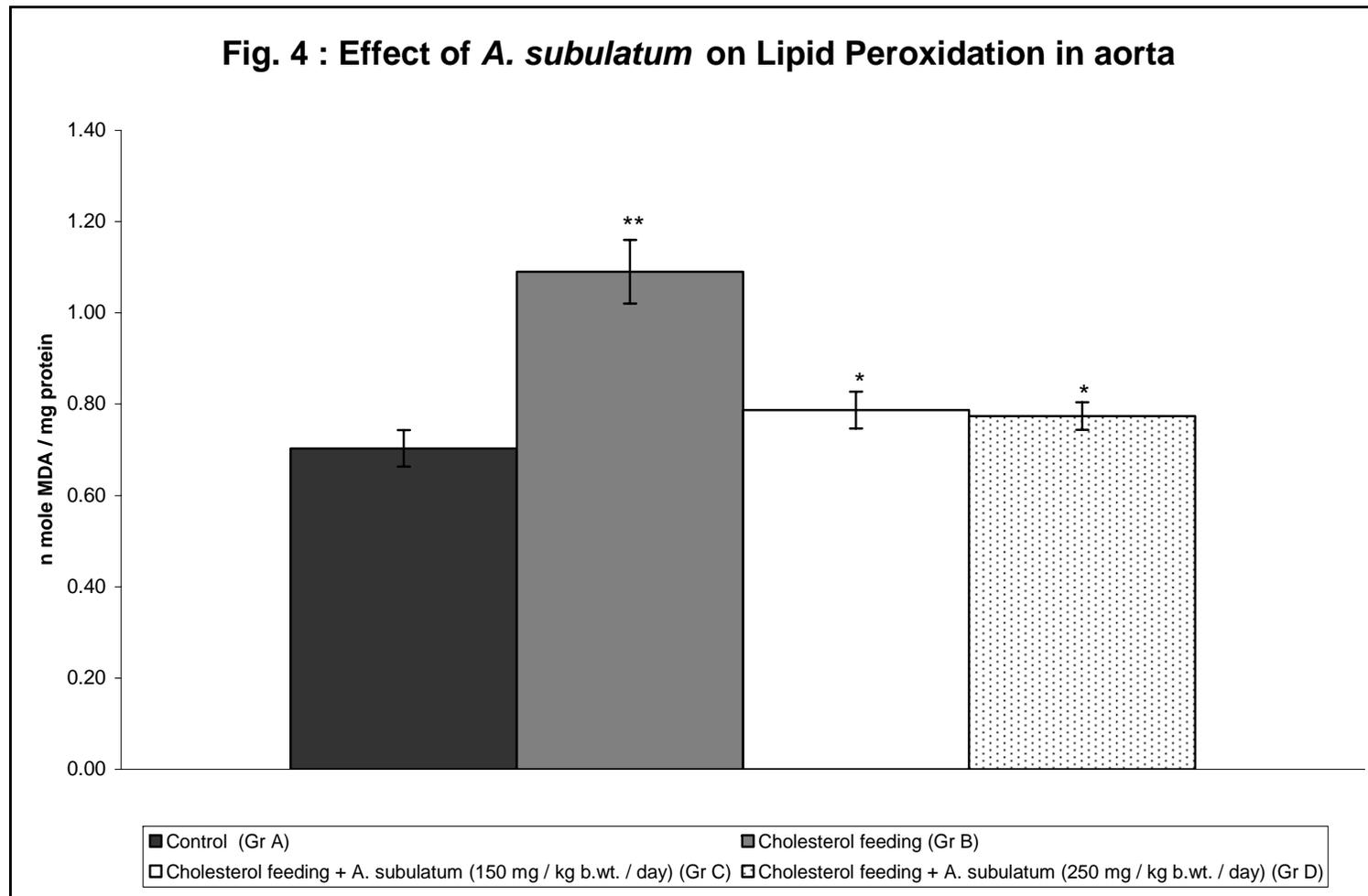
* $P \leq 0.01$ Significant
** $P \leq 0.001$ Highly Significant
ns = non significant

Group B Compared with Group A
Group C and D compared with Group B



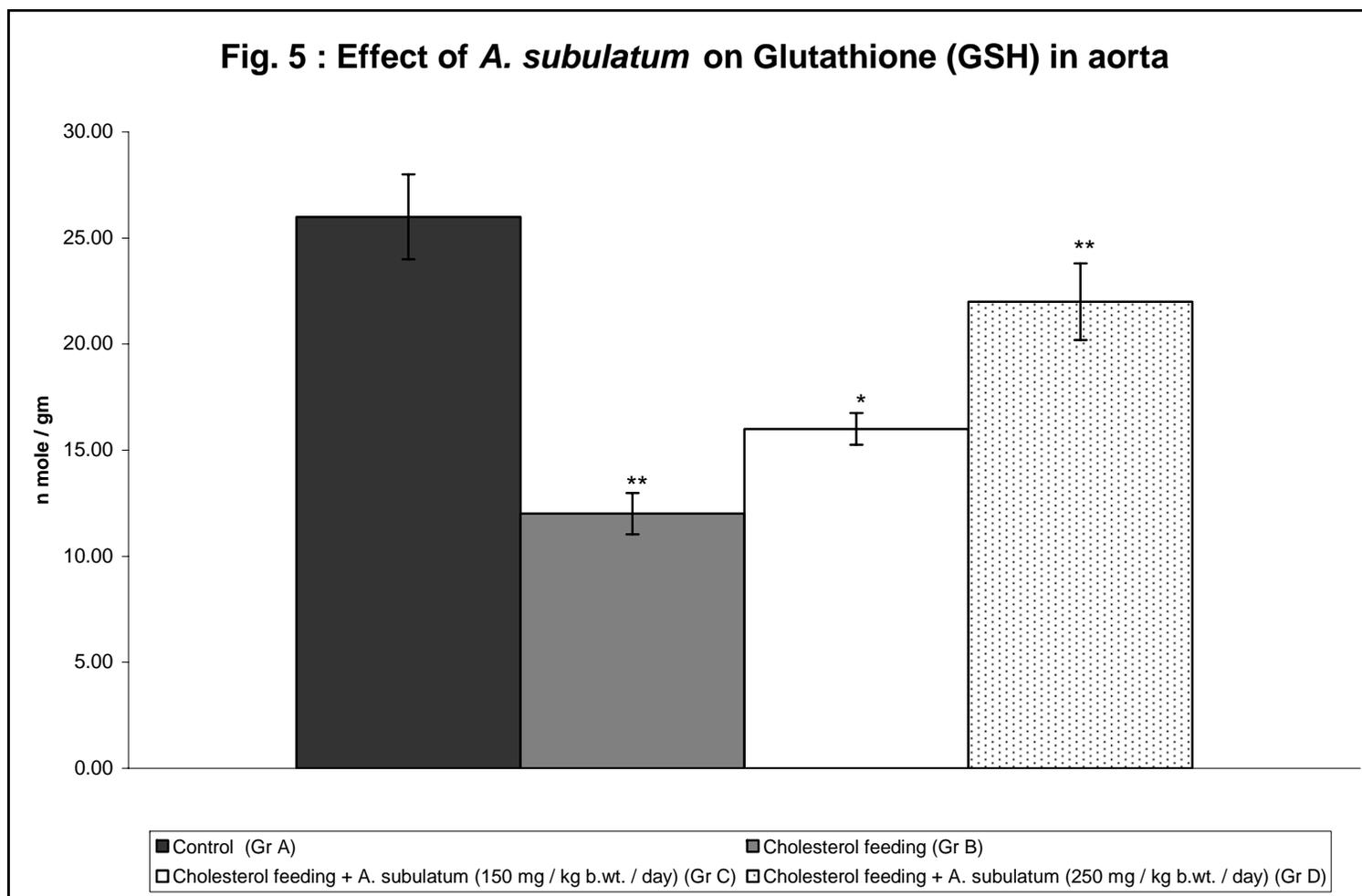
* P ≤ 0.01 Significant
 ** P ≤ 0.001 Highly Significant

Group B Compared with Group A
 Group C and D compared with Group B



* $P \leq 0.01$ Significant
** $P \leq 0.001$ Highly Significant

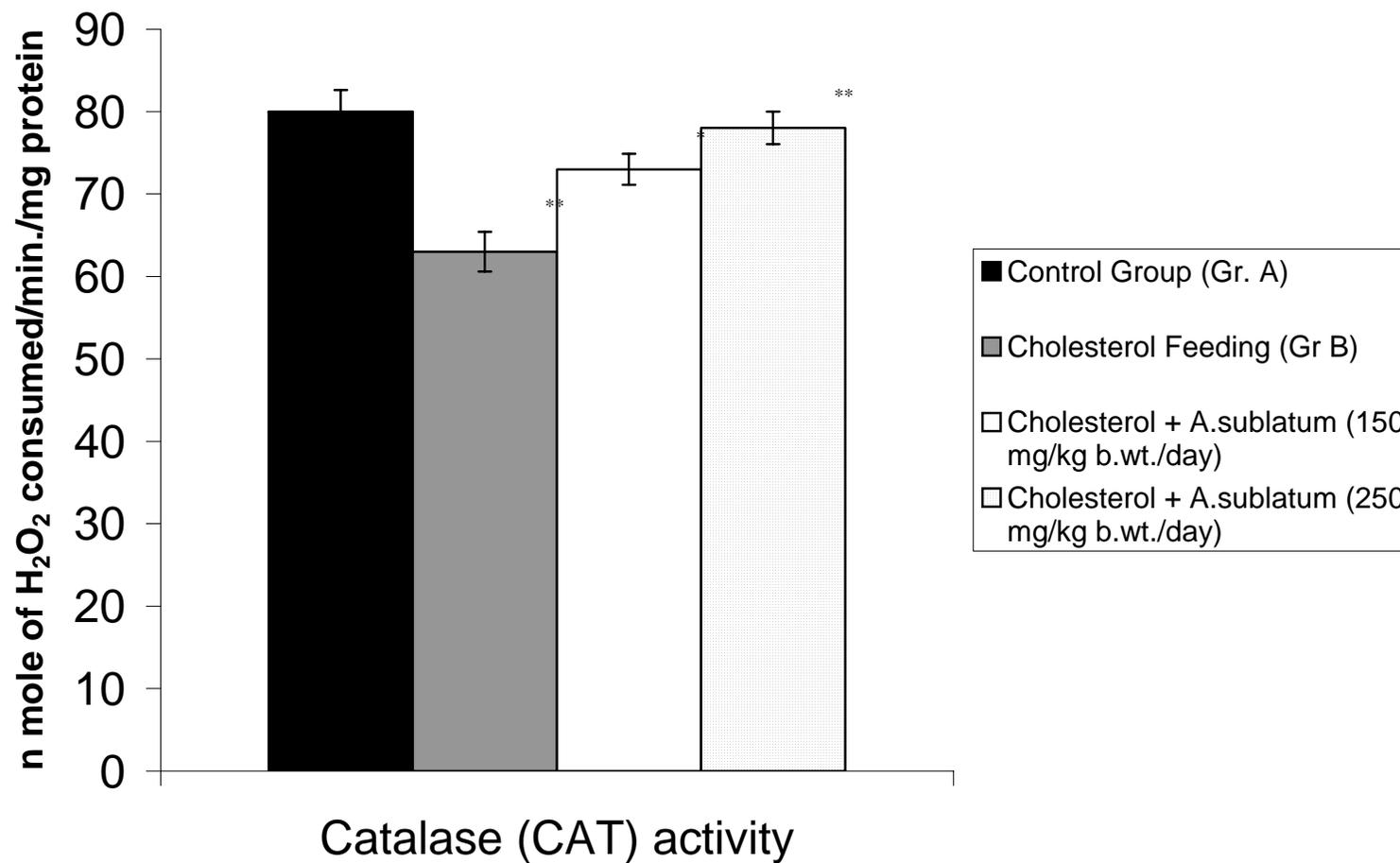
Group B Compared with Group A
Group C and D compared with Group B



* $P \leq 0.01$ Significant
 ** $P \leq 0.001$ Highly Significant

Group B Compared with Group A
 Group C and D compared with Group B

Fig. 6 : Effects of *A. Subulatum* on Catalase (CAT) Activity in aorta



* $P \leq 0.01$ Significant

** $P \leq 0.001$ Highly Significant
Group B

Group B Compared with Group A

Group C and D Compared with