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Comparative Study of Diallyl-Disulphide and Dipropyl-Disulphide in Experimental Atherosclerosis

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Abstract

Diallyl disulphide, the principle organosulphur compound of garlic oil, is known to possess many clinical beneficial effects, but its overuse or abuse has been reported to cause certain harmful side effects due to its possible metabolite acrolein. It was thought that the disulphide nature of diallyl disulphide is responsible for its hypolipidemic effect and the unsaturation may be for its toxic effects. Recently few synthetic disulphides are successfully employed in experimentally induced hyperlipidemia. The present study was under taken to compare the hypolipidemic as well as toxic effects of saturated disulphide, Dipropyl disulphide with Diallyl disulphide. The atherogenic diet fed male albino rats were given orally 100mg/kg body weight of disulphide (DADS or DPDS) for 60 days, later the rats were sacrificed and the plasma lipid profile, glycoproteins, calcium and transaminases were estimated. The aortic homogenates were employed for the estimation of thiobarbituric acid reactive substances and total sulphhydryl group. The results indicate a significant hypolipidemic effect with dipropyl disulphide with a comparative lower toxic side effect. It is concluded that DPDS is much safer and equally good hypolipidemic agent in experimentally induced hyperlipidemia in albino rats.

Index terms— diallyl disulphide, dipropyl disulphide, atherosclerosis, lipid profile, acrolein.

1 Introduction

arlic and its extracts are known to have proved hypolipidemic as well as anti atherosclerotic effects **??** .

The principle organo sulphur compound, Diallyl disulphide (DADS) is thought to be responsible for the hypolipidemic and hypocholesterolemic effects of garlic 2 . However few recent studies have shown that Garlic and DADS May induce certain biochemical toxic effects like increased in blood urea levels, increased plasma transaminases levels 3 as well as increased TBARS production 4 . It was presumed that the disulphide nature of DADS is responsible for its hypolipidemic and hypocholesterolemic effects where as the unsaturation or allyl groups present in DADS may be responsible for its toxic effects. Further a few synthetic disphulphide have been employed with moderate success in regulating hyperlipidemia **??** .

The present study was under taken to compare the hypolipidemic as well as toxic effects of saturated aliphatic low molecular weight disulphide Dipropyldisulphide (DPDS) with Diallyl dispulphide (DADS).

2 II.

3 Materials & Methods

All the chemicals employed in the present study were of Analer (AR) Grade DADS & DPDS were procured from sigma Aldrich Company, USA.

4 a) Atherogenic Diet

The atherogenic diet to feed & to induce atherosclerosis in male albino rats was prepared by mixing whole milk powder, dalda (vegetable ghee) and pure cholesterol in the ratio of 1:0.5:0.1 with an extra vit D 2 supplement of 4 mg/100 g.

5 b) Experimental Animals

Male albino rats of 6 to 8 weeks old weighing 150 g -200 g were selected randomly for the present study from the animal house of Dr. B.R. Ambedkar Medical College Bangalore, upon approval of the committee of ethics in animal experimentation (132/1999/CPSEA). These rats were kept on stock laboratory diet (Amruth rat feed Nava maharatara Chakan oil Ltd. Pune.) and tap water adlibitum. Consisting 6 male albino rats maintained on atherogenic diet adlibitum for 60 days and given 100 mg of DADS as 30 ml warm aqueous solution/kg body weight for 60 days using gastric tube. iv. Group-4 (DADS Curative group) Consisting 6 male albino rats maintained on atherogenic diet adlibitum for 60 days and later given 100 mg of DADS as 30 ml warm aqueous solution/kg body weight daily for next 60 days using gastric tube. During DADS feeding, the rats were maintained on stock laboratory diet, water was provided adlibitum to all these rats always. v. Group-5 (DPDS Protective group) Consisting 6 male albino rats maintained on atherogenic diet adlibitum and were given 100 gm DPDS as 30 ml warm aqueous solution/kg body weight for 60 days using gastric tube.

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vi. Group-6 (DPDS Curative group) Consisting 6 male albino rats maintained on atherogenic diet adlibitum for 60 days and later given 100 mg of DPDS as 30 ml warm aqueous solution/kg body weight daily using gastric tube for next 60 days. During DPDS feeding, the rats were maintained on stock laboratory diet and tap water, water was provided adlibitum to all these rats always. The rats of the group 1.2,3 & 5 were sacrificed by decapitation on the 61 st day and the rats of group-4&6 were sacrificed by decapitation on the 121 st day. Blood samples were collected using heparin as anti coagulant. The blood samples were centrifuged at 3600rpm for 5 minutes, the separated plasma were employed for estimation of total lipids (TL) 5 , triacyl glycerol (TAG) 6 , total cholesterol (TC) 7 phospholipids (PL) 8 HDL cholesterol 9 , free fatty acid (FFA) 10 , esterified fatty acid (EFA) 10 , calcium 11 , glycoprotein 12 , fibrinogen 12 , lipoperotein lipase 13 , aspartate amino transferase (AST) 14 , and alanine amino transferase (ALT) 14 . Aorta was procured and put into a pre weighed dry watch glass.

A portion of aorta was immediately fixed in buffered formalin and was employed for histopathological study.

A second portion of aorta was homogenized with chloroform methanol (1:1v/v) mixture and the extracts were used for estimation of lipid parameters.

7 (TL, TAG, TC & PL).

A third portion of aorta was homogenized with 5% cold TCA and the extracts were used for the estimation of thiobarbituric acid reactive substances (TBARS) 15 .

A fourth portion of aorta was homogenized with phosphate buffer (p H 7.4) and the extracts were used for the estimation of total protein 16 (TP) and total sulphydryl groups 17

8 (SH).

Data obtained were analyzed comparing the results of groups using students 't' test. Probability values less than 0.02 were considered as significant.

IV.

9 Results

Results obtained in the present study are given in table 1 & 2 as well as in figure **??** It is seen from the table-2 there is a significant rise in aortic levels of TL, TAG, TC, PL and TP in control group as compared to normal group suggesting feeding atherogenic diet leads to accumulation of lipids and proteins in aorta. These values are significantly reduced in DADS protective, DADS curative DPDS protective and DPDS curative group establishing that feeding DADS and DPDS decreases the accumulation of lipids in aorta.

The aortic TBARS levels decreased and total SH group -increased in DADS protective, DADS curative, DPDS protective & DPDS curative group as compared to control group as seen from Table **??**.

Figures **??**-6 shows the histopathological findings of aortic cross section (H &E stain) of normal, control, DADS protective, DADS curative, DPDS protective and DPDS curative group of rats. It is evident from table 1 all the lipid parameters except HDL cholesterol are increased in control group as compared to normal group. These parameters were significantly reduced in DADS protective, DADS curative, DPDS protective and DPDS curative group of rats compared to control group establishing both DADS and DPDS has hypolipidemic effects. Further a raise in Glycoprotein and Fibrinogen levels seen in control group as compared to normal group. Whereas feeding DADS & DPDS significantly reduces these values in protective as well as curative group as compared to control groups. The plasma AST and ALT levels are elevated in control group as compared to normal group showing a possibility of tissue damage.

The histological aortic cross section of group 1-6 rats are given in figures 2-6. It is evident from the figures that there is an accumulation of lipids in aortic walls in control group (ref fig- **??**) as compared to normal group (ref fig- **??**). Further there is a significant decrease in this accumulation in both protective (ref fig 3 & 5) as well as curative groups (ref fig 4 & 6) V.

10 Discussion

The optimum dosage of DADS (100 mg/kg body weight) or DPDS (100 mg/kg body weight) employed in the present study clearly establishes the hypolipidemic, hypocholesterolemic and antiatherosclerotic effects of these disulphides. A significant reduction is observed in both plasma and aortic lipids in DADS protective group (group 3), DADS curative group (group 4), DPDS protective group (group 5) and in DPDS curative group (group 6) as compared to antherogeinc diet fed control group (group 2) as evident from the tables 1 & 2. Further it is established by the histological studies of the aortic sections of these group of rats (fig 3-6) that both these disulphides have significant antiatherosclerotic effects in atherogenic diet fed rats (ref fig **??**) . It has been repeatedly established by the earlier workers 18 that garlic has hypolipidemic, hypocholesterolemic and anti atherosclerotic effects 19 and the possible constituent of garlic bringing up this effect is DADS, as it is known that DADS is the principle organo sulphur compound of garlic oil 20 .

Both DADS and DPDS are disulphides and similar to any other disulphide may undergo degradation to their respective thiols utilizing NADPH 21 . This leads to the depletion of cellular available NADPH levels and affects the synthesis of fatty acid, fats and cholesterol as their synthesis requires NADPH 22 hence resulting in a decrease in the plasma and aortic tissue lipid parameters including cholesterol as observed in DADS or DPDS treated atherogenic diet fed rats (group 3, 4, 5 & 6) as compared to control atherogenic diet fed rats (group 2).

HMG CoA reductase is the key enzyme of cholesterol biosynthetic and it is known that DADS has significant inhibition action against this enzyme 19,23 . Through such an inhibition DADS can effect lowering of plasma as well as aortic cholesterol levels as evident from the result given in table 1 & 2. DPDS being a disulphide may induce inhibition of HMG CoA reductase similar to DADS, hence causing a significant lowering of cholesterol levels in plasma & in aorta (refer table **??**.

It is known that disulphide can undergo sulphydrly exchange reaction with tissue proteins and thiol enzymes as depicted below-R -S -S -R + ENZ -SH R -S -S -ENZ + R -SH DADS and DPDS are disulphides and may possibly undergo similar sulphydrly exchange reactions with the tissue proteins as well as thiol enzymes. Such a possible sulphdrly exchange reaction with Fatty acid synthase, HMG CoA reductase, glycerol phosphate dehydrogenase, squalene synthase and squalen oxidase leading to a conformational change in these enzymes resulting in a possible inhibition of these enzymes thereby causing in a significant reduction in fat, fatty acid and in cholesterol synthesis 21 .

The atheromatus plaques in blood vessels are produced by an over accumulation of certain proteins and calcium as well as cholesterol **??**4 . The disulphide, DADS and DPDS significantly lowers the plasma levels of calcium, glycoproteins and fibrinogen in DADS as well as DPDS treated groups (group 3 & 4, group 5 & 6) as compared to control group (group 2). Suggesting that these disulphides promote a decrease in the plasma levels of calcium, glycoprotein's and fibrinogen thereby reduces their accumulation in the intima of blood vessels resulting in showing down of atheromatous plaque formation. This is evidenced by the histological aortic cross section of these rats (ref. Lipoprotein lipase, also known as clearing factor, helps in the clearing of triacylglyerols from plasma. The activity of this enzymes is significantly higher both group 3 & group 4 as compared to group 2 suggesting that both DADS & DPDS improves clearing of plasma triacylglycerols hence favours reduction in plasma / aortic tracylglycerols which is evident from the results given in table 1 & 2 . The disulphide DADS and DPDS might have undergone a sulphydryl exchange reaction with the lipoprotein lipase probably activating the enzyme or increasing the lifespan of the enzyme resulting in a significant reduction in plasma/ aortic triacylglycerol levels.

This observed reduction in plasma and tissue triacylglycerol levels may be in part due to a possible sulphydryl exchange reaction of these disulphides with glycerol phosphate dehydrogenate thus resulting in a partial inhibition of the enzyme leading to a decreased glycerol phosphate formation hence a decreased triacylglycerol production.

The observed in the present study clearly established that DPDS, a saturated, water soluble, well tolerated disulphide has a significant comparable hypolipidemic, hypocholesterolemic and antiatherosclerotic actions in atherogenic diet fed rats (ref. Recently it has been shown by many workers 25 that feeding garlic extracts or garlic oil to experimental K animals do induce certain biochemical abnormalities like increases in blood urea levels increases in serum Bilirubin levels, elevation is serum transaminases 3 etc. Feeding 100mg/kg body weight garlic oil go an overnight fasted rat proved fatal and the cause of death was acute pulmonary edema 3 .These findings of garlic oil attributed to its organosulphur compound, DADS.

The disulphide DADS may undergo catabolism in tissues to give rise to allyl mercaptan which might have converted to acrolein by an unknown mechanism.

11 DADS.

Allyl mercaptan. Acrolein NADPH NADP H S

The toxicity of DADS been evidenced by a significant rise on aortic TBARS levels (ref. [1]

[1]( )K

Figure 1:

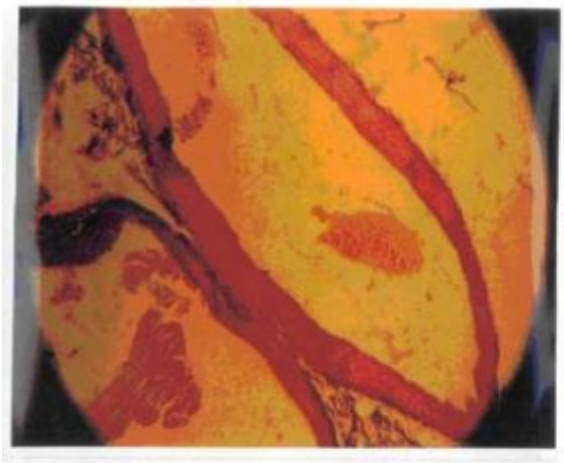

Fig 1 : Normal Group : Shows normal blood vessel/aorta (H & E, x 320)

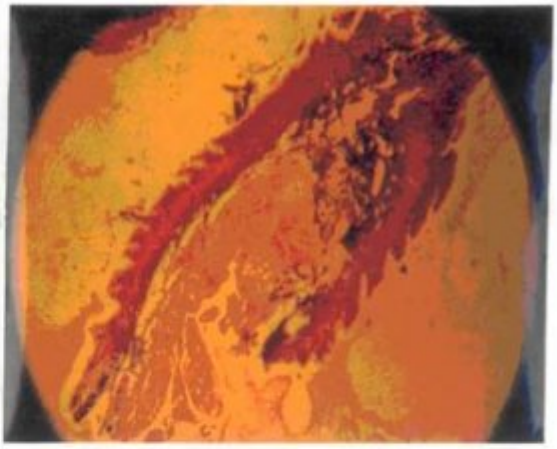

Fig 2 : Control Group (atherosclerotic) : Shows features of atherosclerosis/atheroma with fat deposition. (H & E, x 320)

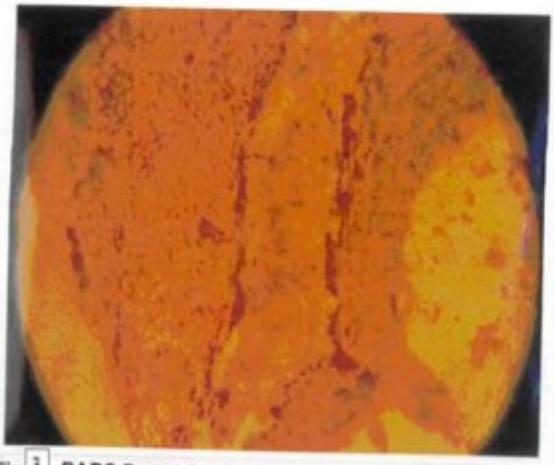

Fig 3 DADS Protective Group : Shows empty fat spaces with blood vessels and few cholesterol clefts and occasionally inflammatory cells. (H & E, x 320)

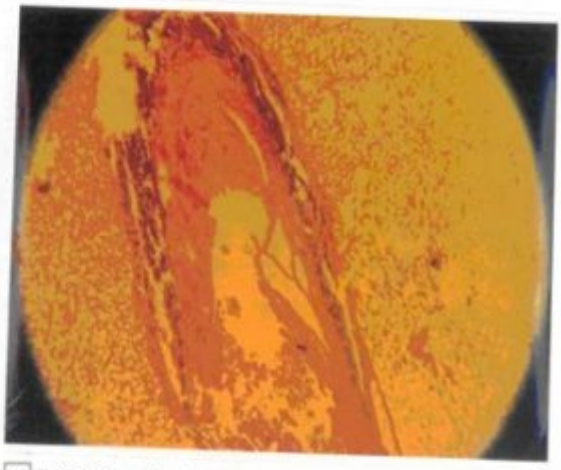

Fig 4 DADS Curative Group : Shows aorta with few cholesterol clefts and fat deposition (H & E , x 320)

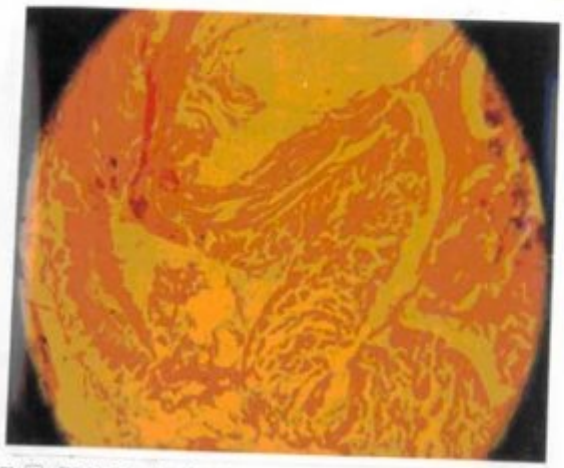

Fig 5 : DPDS Protective Group : Shows normal aorta with mild atherosclerotic changes (H & E, x 320)

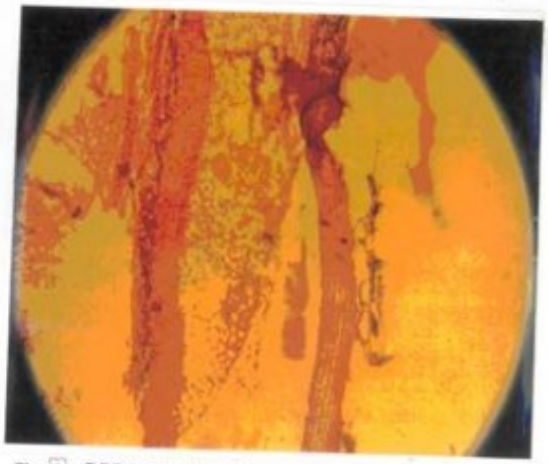

Fig 16 : DPDS Curative Group : Shows wall of the aorta with fatty infiltration (H & E, x 320)

Figure 2:

-6.The plasma levels
of TL, TAG, TC, PL, HDL-cholesterol, EFA, FFA, calcium, glycoprotein, fibrinogen, LPL, AST & ALT in normal group (group 1), control group (group 2), DADS protective group (group 3), DADS curative group (group 4), DPDS protective group (group 5) and DPDS curative group (group 6

Figure 3:

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1, 2 & fig 1-6).

Figure 4: table .

HDL cholesterol (mg%)	6.5±1.43	33.63±0.9	56.3±0.8**	51.6±0.59**	59.1±0.47***	55.2±0.2**
Free fatty acids (Meq/L)	0.312±0.02	0.836±0.02**	0.248±0.02	4.496±0.02***	2.488±0.01	3.504±0.02***
Esterifed fattyacids (mmol/hr)						

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Figure 5:

Table ?? : Table ??howing the plasma levels of TL, TAG, TC, PL, HDL -Cholesterol, FFA, EFA, LPL, Calcium, Glycoprotein Fibrinogen, AST & ALT in normal rats (group-1), in atherogenic diet fed rats (groups-2), in rats fed atherogenic diet and given diallyl disulphide daily (DADS protective group-3), in atherosclerotic rats fed diallyl disulphide daily (DADS curative group-4), in rats fed atherogenic diet and given dipropyl disulphide daily (DPDS protective group-5) and in atherosclerotic rats fed dipropyl disulphide daily(DPDS curative group-6).

[Stephenwarshafsky] , M D Stephenwarshafsky .

[<0] , *** , P <0 . p. 1.

[**p < (0.01)] , **p < . 0.01.

[London Heineman professional publishing Ltd ()] , *London Heineman professional publishing Ltd* 1980. p. 625.

[***] *45** 54.6±0.25** Note: 1. Values are expressed as mean ±SD. 2. No. of animals in each group is 6*, *** . 42.±0.2*** 56.2±0.

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