HYPOGLYCEMIC AND HYPOLIPIDEMIC EFFECTS OF DIACETODIBUTYL DISULPHIDE

P.K. Joseph and R.T. Kasinath*

Department of Biochemistry, St John's Medical College, Bangalore 560 034, India

ABSTRACT

The hyperlipidemia, fatty liver and the high levels of liver and kidney thiobarbituric acid reactive substances (TBARS) observed in rats which were fed ethanol for 45 days, could be significantly reduced by feeding diacetodibutyl disulphide (DADBDS). Ethanol - induced hypoproteinemia and the rise in serum enzymes like AST (EC 2.6.1.10), ALT (EC 2.6.1.2) and ALP (EC 3.1.3.1) could also be ameliorated by DADBDS. Feeding of this compound to normal rats did not produce any change in serum or tissue lipid levels or serum enzymes or tissue TBARS except a moderate reduction in serum triacyl glycerols. DADBDS feeding to rats maintained on a high lipid diet could also reduce the serum and tissue lipid levels and also reduce the serum transaminases.

DADBDS which is an aliphatic disulphide could produce hypolipidemic effects in rats fed a single large dose of ethanol, whereas dimenthol disulphide which is an aromatic disulphide was not useful as a hypolipidemic agent. Perhaps hypolipidemic effects are shown only by aliphatic disulphides and not by aromatic disulphides. Feeding of 100 mg DADBDS per kg body weight to normal fasted rats produced a mild hypoglycemia, but higher doses produced a hyperglycemic effect. This dose of DADBDS increased the serum insulin levels and reduced blood glucose levels in fasted diabetic rats, but DADBDS feeding did not alter the serum insulin levels in fasted normal rats. DADBDS is odourless and tasteless in 1% solution and it could be a better substitute for garlic for hypoglycemic and hypolipidemic studies.

KEY WORDS: Hypoglycemic effect, hypolipidemic effect, DADBDS, garlic

INTRODUCTION

Though garlic possesses hypoglycemic and hypolipidemic properties (1-3), the strong smell and taste are not liked by most people. It is a powerful gastric stimulant and produces some toxic effects at high doses (4,5). The major principles of garlic are diallyl disulphide and smaller quantities of diallyl polysulphides (6). Preliminary studies conducted in our laboratory (unpublished) showed that dipropyl

disulphide has some hypolipidemic effects. But its foul smell and bitter taste made it unsuitable for use. Hence an attempt is made in this paper to synthesise a nontoxic derivative diacetodibutyl disulphide (DADBDS) resembling garlic principle with an acceptable taste and smell.

MATERIALS AND METHODS

Male albino rats 6-8 months old and weighing 200-300 g were divided into groups of six each and used in this study. 2-Mercapto-3-butanol and 8-thio menthol were obtained from Aldrich, USA. All other chemicals used were of the highest purity available and from reputable companies.

Author for correspondence

Dr. P.K. Joseph, Department of Biochemistry
Vinayaga Mission Medical College, Karaikal 609609
Tamilnadu

*Present address: Department of Biochemistry,
Ambedkar Medical College, Bangalore 560 045

Preparation of dimenthol-disulphide (DMDS)

1 N Iodine solution was slowly added to 5, 8-thio menthol, drop by drop till a faint iodine colour persisted. 100 ml ice cold diethyl ether was added. The ether solution was clarified with 10 g anhydrous Na_2SO_4 . The ether was evaporated at 45°C and the resulting compound was used as dimenthol disulphide (DMDS). It was fed as aqueous suspension at 45°C to the animals.

Preparation of diacetodibutyl disulphide (DADBDS)

5 g of 2-mercapto-3-butanol was treated with 1 N iodine solution till a faint colour of iodine persisted. This was mixed with 100 ml ice-cold diethyl ether. It was kept in ice bath and 10 ml acetyl chloride was added and mixed. The mixture was kept at 0°C for 3 hours. The ether layer was separated and washed with 10 ml of 0.1 N-NaOH and with 10 ml distilled water. The ether layer was clarified with about 5 g anhydrous Na_2SO_4 . Ether was evaporated in an open beaker at 45°C . The white crystalline compound obtained was used as diacetodibutyl disulphide.

Hypolipidemic studies using DADBDS and DMDS in rats fed a single large dose of ethanol

30 ml per kg body weight, normal saline was administered intragastrically to control rats of group 1 after 8 hours fasting. Similarly group 2 rats were fed 30 ml 30% ethanol (v/v) per kg body weight, intragastrically, after 8 hours fasting. Group 3 rats were fed the same dose of ethanol mixed with 100 mg DADBDS per kg in the same way. This optimum dose of DADBDS was arrived at in the preliminary studies using 2 rats each, by feeding the same dose of ethanol and different doses of DADBDS. Group 4 rats were also fed the same dose of ethanol mixed with 150 mg DMDS per kg after 8 hours fasting. All these rats were sacrificed by decapitation, 18 hours later, without any further feeding. Water was always provided ad libitum in the bottle. Lipids in the liver and kidneys were extracted using chloroform: methanol (1:1 v/v) mixture (8). Total lipids, triacylglycerols and total cholesterol (9) were estimated in the serum and also in the extracts of liver and kidneys.

Biochemical effects of DADBDS in rats fed ethanol for 45 days

Ethanol fed rats of group 3 in this series, received 30 ml 30% ethanol (v/v) per kg body weight intragastrically for 45 days, Group 4 rats also received the same dose of ethanol mixed with DADBDS at a dose of 100 mg per kg for the same period. Control rats of group 1 were fed similar volume of water every day intragastrically. Group 2 rats were fed the same volume of water mixed with DADBDS at a dose of 100 mg per kg for the same period. All these rats were maintained on stock laboratory diet.

Four hours after the last feeding, the rats were sacrificed by decapitation. Glucose was estimated from a fresh sample of blood (9). Urea, albumin, total proteins, aspartate transaminase (AST) (EC 2.6.1.10) alanine transaminase (ALT) (EC 2.6.1.2) and alkaline phosphatase ALP (EC 3.1.3.1) (9) were estimated in the serum. Triacylglycerols, total cholesterol and total lipids were estimated in the serum and also in the extracts of liver and kidney as in the previous experiment. Thiobarbituric acid reactive substances (TBARS) were estimated in the liver and kidneys (10).

Hypolipidemic studies using DADBDS in rats fed a high lipid diet (HLD)

HLD was prepared by mixing 50 g fat as Dalda, 32 g whole wheat flour and 16 g whole milk powder, 1g NaCl, 1 g cholesterol, 0.2 mg thiamine, 0.25 mg riboflavin and 3 mg niacin were also added to it. Rats of group 2 and 3 in this series were fed ad libitum the HLD for 45 days. Group 1 rats were fed only the stock laboratory diet. Group 3 rats were also fed 100 mg DADBDS per kg as drops down the throat in aqueous suspension for these 45 days. On the 45th day all the rats were sacrificed by decapitation. Total proteins albumin, urea, AST, ALT and ALP were estimated in the serum (9). Glucose was estimated in a fresh sample of blood (9). Triacylglycerols, total lipids and total cholesterol were estimated in the serum and in the extracts of liver and kidneys (9). TBARS were also estimated in the liver and kidneys (10).

Effect of DADBDS on the blood glucose levels in the fasted rats

Six groups of rats were fasted for 24 hours and were fed 0, 50, 100, 200, 300 or 500 mg DADBDS per

2 hours blood was collected from these rats from the tip of the tail by cutting a part of it. Glucose was estimated in these blood samples (9).

Effect of DADBDS on blood glucose and serum insulin levels in normal and in diabetic rats

Rats were injected with 70 mg alloxan in 1.0 ml saline per kg through the tail vein, after 24 hours fasting. They were given food only after another 6 hours. Rats which showed glucose in the urine for 6 consecutive days and having blood glucose 250 - 350 mg per dl, were only used as diabetic rats. 8 hours fasted diabetic rats were fed intragastrically 100 mg DADBDS per kg as aqueous suspension. Diabetic control rats were fed similar volume of water. Similarly a group of normal rats, after fasting for 8 hours were given intragastrically 100mg DADBDS per kg as aqueous suspension. Normal control rats only received similar volume of water intragastrically after 8 hours fasting. 2 1/2 hours later all the rats were sacrificed by decapitation. Glucose was estimated from a fresh sample of blood (9). Serum was used for the radio immunoassay of insulin using the kits supplied by BARC Bombay (11).

Effect of DADBDS on intestinal absorption of ethanol

After 8 hours fasting one group of rats were given 15 ml 30% (v/v) ethanol per kg intragastrically. Another group of rats were fed similar amount of ethanol mixed with DADBDS at a dose of 100 mg per kg. Blood was obtained from both the groups of rats by transretinal bleeding and were immediately used for ethanol estimation after deproteinising (12), at 0, 30, 60, 90, 120 and 150 minutes. DADBDS did not affect intestinal absorption of ethanol.

RESULTS

Hypolipidemic studies using DMDS and DADBDS in rats fed 3 ml 30% ethanol per kg body weight

Ethanol fed rats of group 2 exhibited (Table 1) increase in total lipids of serum, liver and kidneys by 272 % ($P<0.01$), 76 % ($P<0.01$) and 39% ($P<0.05$) respectively, and in liver triacylglycerols, liver total

cholesterol and kidney total cholesterol by 101% ($P<0.01$), 49% ($P<0.01$) and 34% ($P<0.05$) respectively (Table 1). Preliminary studies by feeding different doses of DADBDS in ethanol fed rats showed that 100 mg per kg body weight produced significant hypolipidemic effects and this dose was used in all other studies.

Group 3 rats which were fed the above dose of ethanol and DADBDS, showed a significant reduction of serum total lipids by 34% ($P<0.01$), liver triacylglycerols by 61% ($P<0.01$) serum cholesterol by 53% ($P<0.01$) and liver cholesterol by 54% ($P<0.01$) compared to ethanol fed rats of group 2 (Table 1). When 150 mg DMDS was fed along with ethanol (Group 4), there was a decrease of 20% in serum total lipids ($P<0.05$) and 32% in serum triacylglycerols ($P<0.05$), whereas liver total lipids increased further by 33 % ($P<0.05$) (Table 1). There was no effect on other lipid parameters by DMDS feeding. Preliminary studies showed that lower doses of DMDS did not show hypolipidemic effects and higher doses were too toxic and many animals died in such experiments.

Biochemical effects of DADBDS in rats fed ethanol for 45 days.

Feeding of 100mg DADBDS per kg body weight to normal rats for 45 days (group 2) did not alter any of the parameters studied except for a moderate reduction in serum triacylglycerols ($P<0.01$) (Table 2). Ethanol fed rats of group 3 exhibited an increase of 194%, 1446%, 458% and 79% ($P<0.01$) respectively in urea, AST, ALT, and ALP of serum. They also showed decrease of 11% ($P<0.05$), 38% ($P<0.01$) and 70% ($P<0.01$) in blood glucose, total serum proteins and serum albumin levels. Ethanol fed rats had elevated levels of lipids in serum, liver and kidneys. In serum total lipids, triacylglycerols and total cholesterol were increased by 227%, 178% and 145% ($P<0.01$) respectively. In liver the rise of total lipids, triacylglycerols and cholesterol were 58% ($P<0.01$), 62% ($P<0.01$) and 26% ($P<0.05$) respectively. In kidneys the rise in total lipids triacylglycerols and cholesterol were 33%, 114% and 50% ($P<0.01$) respectively. TBARS of liver and kidneys were also elevated by alcohol feeding by 539% and 167% ($P<0.01$).

Table 1. Hypolipidemic effects of diacetodibutyl disulphide (DADBDS) and dimenthol disulphide (DMDS) in 8 hours fasted rats fed 30 ml, 30% of ethanol.

	Group 1 Control rats fed saline	Group 2 Ethanol fed rats	Group 3 Ethanol + DADBDS fed rats	Group 4 Ethanol + DMDS fed rats
Serum total lipids	3.49±0.50	13.0±1.1**	8.58±1.38**	10.35±0.71*
Liver total lipids g/kg	30.2±2.0	53.1±13.1**	22.2±6.1**	70.6±6.6*
Kidney total lipids g/kg	24.0±2.6	33.3±7.3*	23.3±5.66	37.0±1.41
Serum triacyl glycerols mmol/l	0.68±0.07	0.78±0.12	0.49±0.08**	0.53±0.10*
Liver triacyl glycerols mmol/kg	27.5±3.05	55.3±5.15**	21.8±1.84**	48.3±2.1
Kidney triacyl glycerols mmol/kg	18.7±4.45	25.0±3.64	21.3±4.40	26.2±1.8
Serum total cholesterol mmol/l	1.88±0.15	2.3±0.32	1.08±0.23**	2.14±0.18
Liver total cholesterol mmol/kg	20.4±1.2	30.4±4.07**	14.0±3.29**	28.1±1.83
Kidney total cholesterol mmol/kg	16.0±2.31	21.4±2.51*	16.8±3.16	17.9±0.80

Results are expressed as mean±SD. Number of rats in each group is 6. Group 2 is compared to group 1, group 3 and 4 compared to group 2. Statistics calculated using students 't' test.

*p<0.05; +p<0.02; **p<0.01

When 100 mg per kg body weight of DADBDS was fed along with ethanol for 45 days to group 4 rats, all the lipid levels in serum liver and kidneys and serum enzymes showed significant reduction (Table 2) compared to ethanol fed rats of group 3. In serum AST, ALT, ALP, total lipids triacylglycerols and total cholesterol decreased by 62%, 60%, 88%, 32%, 65% and 41% respectively (P<0.01) (Table 2).

In group 4 rats serum total proteins and serum albumin increased by 52% and 188% (P<0.01). DADBDS feeding to ethanol fed rats did not alter serum urea level (P>0.1) and reduced blood glucose by 25% (P<0.05). DADBDS feeding also reduced the TBARS in liver and kidneys by 49% and 33% (P<0.01) (group 4) (Table 2).

However, DADBDS could not restore the above parameters to normal levels in ethanol fed rats (group 4). In serum the total lipids and total cholesterol remained elevated by 123% and 43% respectively (P<0.01) in group 4 rats compared to control rats of group 1. TBARS in liver and kidneys in these rats (group 4) were 225% and 167% higher (P<0.01).

while blood glucose was 33% less (P<0.01) compared to control rats of group 1.

Effects of feeding HLD to rats for 45 days

Hyperlipidemic effects induced by feeding HLD for 45 days (group 2) also increased serum AST by 218% (P<0.01) (Table 3). The rise in total lipids, triacylglycerols and total cholesterol observed in serum were 112%, 183% and 292% (P<0.01) respectively. In liver 322%, 409% and 156% (P<0.01) and in kidneys 62%, 44% and 68% (P<0.01) increase were observed in total lipids, triacylglycerols and total cholesterol respectively.

When HLD fed rats were also given 100 mg DADBDS per kg body weight (Group 3) significant reduction of lipid levels of serum liver and kidneys and serum AST were observed compared to HLD fed rats of group 2. The decrease of total lipids, triacylglycerols and total cholesterol observed in serum were 20%, 34% and 51%, in liver were 31%, 69% and 45% and in kidneys were 35%, 24% and 33% respectively (P<0.01). But this dose of DADBDS could not restore the lipid levels and serum

Table 2. Biochemical effects of feeding 100 mg DADBDS per kg body weight to normal rats and in rats fed 30 ml 30% ethanol per kg for 45 days during the same period.

	Group 1 Saline fed control rats	Group 2 Control rats fed DADBDS	Group 3 Rats fed ethanol for 45 days	Group 4 Rats fed ethanol + DADBDS for 45 days
Blood glucose mmol/l	3.20±0.2	2.60±0.20*	2.86±0.18*	2.15±0.34*
Serum urea mmol/l	2.20±0.10	1.90±0.90	6.47±1.36**	6.64±1.56
Serum AST IU/l	67.9±14.4	63.2±14.4	1050±99**	404±65.6**
Serum ALT IU/l	61.4±11.1	45.6±10.4	343±62**	137±53**
Serum ALP KAU	44.8±8.0	41.6±9.6	400±52.8**	48±22.4**
Serum total proteins g/l	65.0±5.4	64.2±6.3	40.1±12.0**	61±13.0**
Serum albumin g/l	35.0±3.5	34.8±2.8	10.2±6.0**	29.4±5.0**
Serum total lipids g/l	3.68±0.5	3.00±0.6	12.05±0.9**	8.2±0.8**
Serum triacyl glycerols mmol/l	0.69±0.07	0.45±0.04**	1.92±0.09**	0.67±0.06**
Serum total cholesterol mmol/l	1.80±0.16	1.60±0.12	4.41±0.24**	2.57±0.21**
Liver total lipids g/kg	30.7±2.5	27.2±2.4	57.9±7.4**	21.6±4.1**
Liver triacyl glycerols mmol/kg	29.5±3.0	27.4±4.1	61.9±8.2**	33.5±5.8**
Liver total cholesterol mmol/kg	20.4±2.3	16.7±2.1	25.9±4.2*	16.8±3.9**
Kidney total lipids g/kg	22.2±3.5	21.6±3.8	29.6±3.2**	19.0±4.8**
Kidney triacyl glycerols mmol/kg	18.0±4.2	18.2±1.2	38.68±6.8**	27.0±5.4*
Kidney total cholesterol mmol/kg	16.7±3.3	14.2±2.1	25.1±3.5**	16.3±4.2**
Liver TBARS U moles/g	6.80±2.1	5.80±1.2	43.5±8.6**	22.1±2.8**
Kidney TBARS	8.60±1.3	5.30±2.4	23.0±6.4**	15.3±4.3**

Results are expressed as Mean±SD. Number of animals in each group is 6. Group 2 and 3 are compared with group 1 and group 4 is compared with group 3. Statistics calculated using students 't' test. *p<0.05; +p<0.02; **p<0.01

AST (group 4) to the levels seen in normal rats of group 1. DADBDS feeding (group 3) reduced serum AST by 35% and blood glucose by 25% (P< 0.01) in HLD fed rats.

Group 3 rats had 70%, 88% and 91% higher total lipids, triacylglycerols and total cholesterol in the serum and 155%, 59% and 40% higher levels of these parameters in liver respectively compared to normal rats of group 1 (P<0.01). Liver and kidney TBARS in group 3 rats were not significantly different compared to normal rats of group 1.

Effect of DADBDS on intestinal absorption of ethanol

Blood ethanol levels of rats fed ethanol and DADBDS were never lesser than those of rats fed ethanol alone and both the levels were some what similar (Fig. 1).

Effect of various doses of DADBDS on blood glucose levels in fasted rats

Feeding of 100 mg DADBDS per kg produced the maximum hypoglycemic activity at 2½ hours

Table 3. Hypolipidemic effects of feeding 100 mg DADBDS per kg body weight to high lipid diet (HLD) fed rats.

	Group 1 Saline fed control rats	Group 2 rats fed HLD for 45 days	Group 3 rats fed HLD + DADBDS 45 days
Blood glucose mmol/l	3.2±0.3	3.6±0.20	2.7±0.08**
Serum urea mmol/l	2.4±0.21	2.7±0.20	2.8±0.21
Serum AST IU	65.01±12.4	207.4±35.2**	134±25.6**
Serum ALT IU	64.8±12.5	65.6±13.8	100.2±39.2
Serum ALP KAU	41.6±6.3	51.2±5.6*	52.2±19.0
Serum total proteins g/l	66.6±6.3	65.3±5.4	67.3±3.6
Serum albumin g/l	36.3±4.1	30.6±4.1	35.1±1.8
Serum total lipids g/l	3.53±0.50	7.49±1.06**	5.99±0.93**
Serum triacyl glycerols mmol/l	0.68±0.08	1.93±0.25**	1.28±0.26**
Serum total cholesterol mmol/l	1.91±0.15	7.5±1.6**	3.65±0.60**
Liver total lipids g/kg	30.3±2.6	128±14.1**	77.4±19.8**
Liver triacyl glycerols mmol/kg	27.5±3.1	140±17.4**	43.6±4.37**
Liver total cholesterol mmol/kg	21.0±3.1	53.8±5.03**	29.5±5.62**
Kidney total lipids g/kg	22.8±3.1	36.9±5.4**	24.1±2.7**
Kidney triacyl glycerols mmol/kg	18.9±3.9	27.2±5.4**	20.7±3.6**
Kidney total cholesterol mmol/kg	17.0±2.4	28.5±4.1**	19.2±2.7**
Liver TBARS U moles/g	6.8±2.7	14.1±1.4**	8.0±2.4**
Kidney TBARS µmoles/g	7.9±2.1	12.6±1.8**	8.6±2.1**

Results are expressed as Mean±SD. Number of animals in each group is 6. Group 2 is compared with group 1 and group 3 is compared with group 2. Statistics calculated using students 't' test.

*p<0.05; **p<0.01

Table 4. The effects of feeding 100 mg DADBDS per kg body weight to 8 hours fasted normal and diabetic rats on blood glucose and serum insulin levels.

	Serum Insulin Units/l	Blood glucose mmol/l
Control rats fed saline	14.38±2.1	3.0±0.30
Control rats fed DADBDS	16.73±4.85	1.86±0.27*
Diabetic rats fed saline	8.81±1.42	11.32±0.93*
Diabetic rats fed DADBDS	24.8±2.2	5.7±0.60

Results are expressed as Mean±SD. Number of animals in each group is 6. Statistics calculated using students 't' test.

*p<0.001.

in 24 hour fasted rats, as compared to untreated fasted (Fig. 2). Feeding of 300 mg or above of DADBDS produced a hyperglycemic effect as compared to untreated control rats.

Fig. 1. Effect of DADBDS on intestinal absorption of ethanol.

Fig. 2. Blood glucose levels at 2½ hrs after feeding DADBDS to 24 hrs fasted rats.

Effect of feeding 100 mg DADBDS on blood glucose and serum insulin levels in normal and in diabetic rats.

Though this dose of DADBDS produced a significant hypoglycemic effect in 8 hours fasted rats

(61%) ($P < 0.01$), it did not produce any significant increase in serum insulin levels ($P < 0.01$) (Table 4). However this dose of DADBDS had a marked hypoglycemic effect (50%) ($P < 0.01$) in diabetic rats, associated with a similar rise in serum insulin levels (181%) ($P < 0.01$).

DISCUSSION

Feeding of DADBDS at 100 mg/kg body weight produced a reduction of blood glucose levels, but no severe hypoglycemia was observed (Table 4). When 300mg/kg body weight or more was fed to fasted normal rats only, hyperglycemia was produced. Probably the optimum dose of it may be 100mg/kg body weight in rats and this compound may not produce severe hypoglycemia even at higher doses in rats. Feeding of garlic oil at 100 mg/kg body weight to rats increased serum AST, ALT, ALP and TBARS of liver and kidneys (4, 5, 7). In the present series of experiments the same dose of DADBDS when fed to normal rats did not alter any of these parameters (Table 2) unlike garlic. DADBDS is an aliphatic disulphide similar to diallyl disulphide of garlic. The hypoglycemic and hypolipidemic effects may be due to the disulphide group in both the compounds. The allyl group of diallyl disulphide of garlic could be responsible for the changes in serum enzymes and tissue TBARS levels at a dose of 100 mg/kg fed to rats which is not shown by DADBDS which has no allyl group. DMDS which is an aromatic disulphide did not show much hypolipidemic effect (Table 1) where as DADBDS which is an aliphatic disulphide produced some good hypolipidemic effect in rats fed a single large dose of ethanol. Thus the hypolipidemic effects are shown by aliphatic disulphides and not by aromatic disulphides.

DADBDS has a better taste and smell compared to garlic oil and exhibit hypoglycemic and hypolipidemic effects. But the present dose of 100 mg/kg body weight could not fully prevent the hyperlipidemic effects in ethanol fed and BLD fed rats. Probably prolonged treatment or higher dose of this compound could give better results. Perhaps an aliphatic disulphide with a different side chain may prove more useful.

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