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Experimental Research

Biochemistry Section

Effect of Diallyl Disulphide on Diabetes Induced Dyslipidemia in Male Albino Rats

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ABSTRACT

Background: Diabetes Mellitus is a chronic metabolic disorder which may lead to various complications, the important being dyslipidemia leading to Coronary Heart Disorders (CHD), the major cause for morbidity and mortality in diabetic patients. Diabetes Mellitus could be treated by nutritional therapy/drug therapy and others. But the drug therapy would have its own limitations and side effects. To overcome from this an herbal extract is recommended, such as Diallyl Disulphide (DADS) a principle compound of Garlic oil.

Aim: To assess the hypolipidemic effect of Diallyl Disulphide (DADS) in alloxan induced diabetic rats.

Materials and Methods: Healthy adult wistar strain male albino rats weighing around 100-150 grams were randomly selected from the animal house at BLDE University's Shri B.M.Patil Medical College, Hospital and Research Centre, Bijapur, India. Diabetes was induced using alloxan and was treated with DADS. After a stipulated time the rats were anesthetised and sacrificed to collect the blood and liver tissue. Various Lipid parameters, HMG CoA Reductase, Fecal bile acids were estimated in the blood, feces and homogenised liver tissue using standard procedures.

Statiscal Analysis: One-way ANOVA followed by post-hoc t-test is done.

Result: There was significant decrease in the blood and liver tissue lipid parameters of DADS treated alloxan induced diabetic rats when compared to the alloxan induced diabetic rats.

Conclusion: From this study it can be concluded that the DADS a principle compound of garlic, definitely has the hypolipidemic effect in diabetic rats, which is reducing the morbidity in diabetic cases due to dyslipidemia without the adverse effects.

Keywords: Diabetes mellitus, Dyslipidemia, HMG CoA Reductase

INTRODUCTION

Diabetes Mellitus (DM) is a variable disorder of carbohydrate metabolism characterized by reduced insulin secretion (or) decreased glucose utilization [1]. Diabetes Mellitus may lead to many acute and chronic complications. Acute complications include diabetic ketoacidosis and hyperosmolar hyperglycaemic state. Chronic complications include coronary heart disorder, dyslipidemia, retinopathy, nephropathy and neuropathy [1]. Dyslipidemia is one of the major complications associated with DM [2]. If the lipid levels are not controlled may lead to coronary heart disorder [3].

Most diabetic patients start the treatment with diet and exercise but, unfortunately most patients are unsuccessful in controlling diabetes through life style change alone and require drug therapy [4]. Drugs like Biguanides, Sulfonylureas, Thiazolidines, Statins are some of the first medications used in the treatment of diabetes. These drugs have got beneficial as well as adverse side effects [4,5]. In the long run the adverse side effects outweigh the benefits of these drugs. Biguanides reported gastrointenstinal upset and lacticacidosis, Sulfonylureas have the risk of hypoglycemic effect, Thiazolidines are associated with hepatotoxicity [4] and Statins have the risk of myositis, myalgia, liver damage [6].

To substantiate the adverse side effects of these drugs many medicinal plants are in use, which have the hypoglycemic and hypolipidemic activities [7,8]. One among them is garlic (*Allium sativum linn*) known for its anti-hyperglycemic, anti-hyperlipidemic, anti-atherogenic properties [9,10] and many of these properties were attributed to the principle sulphur compound of garlic: Diallyl Disulphide (DADS). Few of the earlier studies fail to confirm the hypolipidemic effect of garlic [11,12]. The present study was under taken to determine the hypolipidemic effect of DADS in alloxan induced diabetic rats.

MATERIALS AND METHODS

Sigma Aldrich chemicals have supplied, Alloxan and Diallyl disulphide (DADS) required for the study. Healthy wistar strain male albino rats weighing around 100-150 gm were randomly selected from the animal house, BLDE University's, Shri B.M.Patil Medical College, Hospital and Research Centre, Bijapur, India, were used for the present study. The experiments were conducted in accordance with Committee for the purpose of Control and Supervision of Experimental Animals (CPCSEA), New Delhi and Institutional Animal Ethical Committee (IAEC) of Shri B.M.Patil Medical College, Hospital and Research Centre, Bijapur, India. These animals were divided into four groups of six rats in each group. Group I: Normal Control, Group II: Diabetic Control, Group III: DADS treated Normal rats, Group IV: DADS treated Diabetic rats. Group I and II rats are given 3ml of normal saline per kg body weight through gastric intubation for 30 days, stock lab diet and water was provided ad libitum. Group III and IV are given 100mg/kg body weight of DADS as 3ml of suspension per kg body weight through gastric intubation for 30 days, stock lab diet and water was provided ad libitum [13]. The study was conducted during the period July 2013 to November 2014.

Induction of diabetes: Induction of diabetes was done by intraperitoneal injection of freshly prepared aqueous alloxan monohydrate (150 mg per kg body weight). in sterile water to overnight fasted rats [14]. Later stock lab diet and water was provided ad libitum. The urine of the rats, which showed positive for sugar after alloxan treatment for 3 consecutive days, was labelled as diabetic rats. A day prior to animal scarification, each animal was kept in a separate cage and the fecal material of each animal was collected. Bile acids were extracted from this fecal material as per procedure [15] and the Bile acids content were estimated to assess cholesterol break down to bile acids.

On the completion of stipulated period, rats were anaesthetised and sacrificed. Blood was collected in heparinised tubes. Liver tissue was procured, then smoothly blotted it to dry, weighed and kept in clean dry beakers covered with aluminium foil.

Blood samples were employed for estimation of various lipid parameters - total lipids [16], total cholesterol [17], triacylglycerols [18], phospholipids [19], HDL cholesterol [17], free fatty acids [20], esterified fatty acids [21], total fatty acids, VLDL cholesterol and LDL cholesterol. One gram of the liver tissues was homogenized with 10ml of chloroform-methanol 1:1 (v/v) and centrifuged. The supernatant of chloroform-methanol extract was used for the estimation of lipid parameters - total lipids [16], total cholesterol [17], triacylglycerols [18] and phospholipids [19]. One gram of the liver tissue was homogenized with 10ml of phosphate buffer (pH-7.4) and centrifuged. The supernatant of phosphate buffer extract was employed for the estimation of HMG Co-A reductase activity [22].

Gravimetry

The body weight of all the animals of each group was recorded on the day 1 of the treatment and on the day of sacrifice. The liver weight was determined after dissecting out and blotting it dry in a single pan balance to evaluate the hepato - somatic index. Hepato - somatic index is the ratio of liver weight after dissection to body weight at the time of sacrifice.

STATISTICAL ANALYSIS

All the results are expressed as mean ± standard deviation. The statistical analysis was done using one-way analysis of variance (ANOVA) followed by post-hoc t-test to determine the significant difference between the groups. A p-value less than 0.005 were selected as the point of minimal statistical significance.

RESULTS

The results of the experiments conducted to assess the Diallyl Disulphide (100mg/kg body weight) induced changes in gravimetry, plasma and liver tissue lipid levels and cholesterol turnover are given in [Table/Fig-1-3].

Results show a significant decrease in final body weight and increase in hepato-somatic index in diabetic rats compared to normal controls. These changes were reversed by the administration of DADS to diabetic rats to [Table/Fig-1].

The plasma and liver lipids are significantly increased in diabetic rats compared to normal controls. DADS administration shows a hypolipidemic effect in diabetic rats to [Table/Fig-2]. [Table/Fig-3] depicts the cholesterol turnover, HMG CoA/Mevalonate ratio and fecal bile acids are significantly decreased in diabetic rats compared to normal controls. DADS administration has improved the HMG CoA/Mevalonate ratio and fecal bile acids in diabetic rats. DADS administration in normal rats has no significant effect in all the parameters when compared to normal controls [Table/Fig-1-3].

DISCUSSION

Dyslipidemia is a major complication associated with high rate of morbidity and mortality in diabetic patients [2]. Increase in plasma and liver lipids in alloxan diabetic rats was shown earlier by CS Yadav [23,24], the results depicted in [Table/Fig-2] agree with this and there is increase in plasma and liver lipids. This complication in diabetic patients can be treated with life style change but unfortunately most patients are unsuccessful in controlling through life style modification alone and require drug therapy [4], which have adverse side effects [4,7]. To substantiate the adverse effects of drugs, DADS a principle organosulphur compound of garlic is employed for its hypolipidemic effects, but the earlier works fail to confirm the hypolipidemic effect [11,12]. This inconsistency may be due to methodological shortcomings, such as mode of administration, short duration, and inadequate statistical power. In the present study feeding of 100 mg/kg body weight of DADS as 3 ml suspension for 30 days through gastric intubation shows a significant hypolipidemic and hypocholesterolemic effect [Table/Fig-2]. The results are in consideration with the earlier studies of hypolipidemic effect of DADS [25].

The most health concern lipid: Cholesterol is a steroid mainly synthesized in the liver from starting material acetyl CoA through a series of reactions regulated by the key enzymes HMG CoA

S.No	Parameter	Group I (n=6)	Group II (n=6)	Group III (n=6)	Group IV (n=6)	f-value	p-value
1	Initial Body weight (g)	260±9 [a]	257±16.8 [a]	251.5±6.9 [a]	253.5±9.3 [a]	0.7444	0.5388
2	Final Body weight (g)	276.6±11 [a]	238v18.1 [b]	266.6±8.1 [a]	242±7 [b]	15.38	<0.0001
3	% Body weight Change	6±1.34 [a]	-8.23±2 [b]	5.6±0.9 [a]	-4.7±1.8 [c]	120.3	<0.0001
4	Hepato-Somatic Index (g/Kg)	29±0.49 [a]	36± 1.2 [b]	30.5±1.8 [a]	33.±0.7 [c]	33.27	<0.0001

[Table/Fig-1]: Gravimetry: Changes in body weight and Hepato - Somatic index in Normal and Alloxan induced Diabetic rats before and after treatment with DADS (100mg/kg body weight) for 30 days
Note: Each value is mean ± SD of 6 observations in each group. In each row values with different superscripts (a,b,c) are significantly different from each other (p<0.05)

S.No	Parameter	Group I (n=6)	Group II (n=6)	Group III (n=6)	Group IV (n=6)	f-Value	p-Value
1	Total Lipids(mg/dl)	164± 18.8[a]	265±46.8 [b]	163.5±21.08 [a]	203.6±23.67[c]	14.83	<0.0001
2	Triacylglycerols(mg/dl)	60±4.0 [a]	90±9.1 [b]	49.8±6.3 [c]	64±7.4 [d]	33.09	<0.0001
3	Total Cholesterol(mg/dl)	58±4.5 [a]	100±6.1 [b]	53±4.8 [a]	77±5.41 [c]	89.33	<0.0001
4	Phospholipids(mg/dl)	31±4.1 [a]	66.4±3.8 [b]	30.5±3.2 [a]	42.6±5.3 [c]	83.24	<0.0001
5	Free Fatty acids(mg/dl)	4.66±0.64 [a]	6.96±0.61 [b]	4.4±0.3 [a]	5.8±0.39 [c]	26.35	<0.0001
6	Esterified Fatty acids(mg/dl)	65±9.74 [a]	90.2±9.39 [b]	68±16.1 [a]	82±12.1 [b]	5.149	0.0090
7	Total Fatty acids(mg/dl)	69.75±9.2 [a]	97.16±9.26 [b]	72.4±16.1 [a]	87.8±11.82 [b]	6.335	0.0037
8	HDL Cholesterol(mg/dl)	35±5.08 [a]	19±2.3 [b]	27.5±4.96 [c]	24±7.64 [b]	8.470	0.0009
9	LDL Cholesterol(mg/dl)	10.3±7.2 [a]	63±5.9 [b]	15.5±2.2 [a]	40.4±5.38 [c]	105.7	<0.0001
10	VLDL Cholesterol(mg/dl)	12.0±0.8 [a]	18.0±1.8 [b]	9.9±1.2 [c]	12.8±1.4 [d]	33.09	<0.0001
	Tissue						
1	Total Lipids(mg/g)	20±2.3 [a]	51±6.9 [b]	19.6±6.7 [a]	30±4.16 [c]	42.17	<0.0001
2	Triacylglycerols(mg/g)	14±1.7 [a]	44±2.9 [b]	12.8±3.4 [a]	32±8.2 [c]	53.33	<0.0001
3	Total Cholesterol(mg/g)	4.3±0.3 [a]	6.1±0.46 [b]	3.8±0.52 [a]	4.0±0.32 [c]	34.67	<0.0001
4	Phospholipids(mg/g)	0.96±0.21 [a]	2.48±0.23 [b]	1.0±0.2 [a]	1.55±0.36 [c]	32.90	<0.0001

[Table/Fig-2]: Changes in lipid profile in normal and alloxan induced diabetic rats before and after treatment with DADS (100mg/kg body weight) for 30 days
Note: Each value is mean ± SD of 6 observations in each group. In each row values with different superscripts (a,b,c,d) are significantly different from each other (p<0.05)

S.No	Parameter	Group I (n=6)	Group II (n=6)	Group III (n=6)	Group IV (n=6)	f-value	p-value
1	Total Cholesterol (p)(mg/dl)	58±4.5 [a]	100±6.1 [b]	53±4.8 [a]	77±5.41 [c]	89.33	<0.0001
2	Total Cholesterol (t)(mg/g)	4.3±0.3 [a]	6.1±0.46 [b]	3.8±0.52 [a]	4.0±0.32 [c]	34.67	<0.0001
3	Liver HMG CoA / Mevalonate Ratio	4.5±0.96 [a]	3.78±0.61 [a]	4.83±1.4 [a]	7.8±1.00 [b]	16.35	<0.0001
4	Fecal Bile Acids (mg/24 hrs fecal matter)	1.2±1.28 [a]	0.67± 0.11 [b]	1.3±0.17 [a]	2.45±0.39 [c]	42.97	<0.0001

[Table/Fig-3]: Changes in cholesterol turnover in normal and alloxan induced diabetic rats before and after treatment with DADS (100mg/kg body weight) for 30 days
Note: Each value is mean ± SD of 6 observations in each group. In each row values with different superscripts (a,b,c) are significantly different from each other (p<0.05)

reductase [26,27]. Cholesterol, so synthesised is mainly utilized for synthesis of bile acids apart from being converted to other useful products in the body [28]. The bile acids – includes primary bile acids – cholic acid, chenodeoxycholic acid and secondary bile acids - deoxycholic acid and lithocholic acid formed by the action of intestinal microflora. The secondary bile acids are excreted through the fecal material. The amount of bile acids excreted in the feces substantiates the amount of the cholesterol utilized through the bile acid pathway in liver. Hence, to assess the cholesterol turnover in the liver, in the present study, the liver tissue total cholesterol content, the rate of activity of the enzyme HMG CoA reductase and the bile acid content of 24 h fecal material was carried out. HMG CoA reductase activity calculated as the ratio of HMG CoA/ Mevalonate and an increase in the ratio indicates lower activity where as a lowered ratio suggests an increased activity. A significant raise in HMG CoA reductase activity and decrease in fecal bile acids was observed in Group II when compared to Group I. The HMG CoA reductase activity and fecal bile acids are reversed by DADS administration in diabetic rats.

CONCLUSION

From the findings of the present study it may be concluded that feeding of 100 mg/kg body weight of DADS for 30 days has hypolipidemic and hypocholesterolemic effect by inhibiting the activity of HMG CoA Reductase a key enzyme in cholesterol biosynthesis (Decreased synthesis) and by increasing the excretion of fecal bile acids, an only route for cholesterol excretion (Increased utilization) in alloxan induced diabetic rats.

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