

Effect of Diallyl Disulphide in Alloxan Induced Diabetic Male Albino Rats

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Abstract

Background: Diabetes Mellitus is a syndrome characterised by a loss of glucose homeostasis from defective insulin secretion and its action; both are resulting in impaired metabolism of carbohydrate, lipid and protein. Diabetes mellitus, if uncontrolled, leads to many complications and the significant being atherosclerosis. Diabetes Mellitus could be treated by nutritional therapy/drug therapy and others. But the drug therapy would have its limitations and side effects. To overcome this, a herbal extract is recommended, such as Diallyl Disulphide (DADS) a principle compound of Garlic oil. **Objectives:** To assess the hypoglycaemic and hypolipidemic effects of Diallyl Disulphide (DADS) in alloxan-induced diabetic rats. **Methods:** Healthy adult Wister 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 anaesthetised and sacrificed to collect the blood. Blood glucose and serum lipid profile were estimated using standard procedures. One way ANOVA followed by post hoc 't' test was done. **Results:** There was a significant decrease in the blood glucose, and lipid parameters of DADS treated alloxan-induced diabetic rats when compared to the alloxan-induced diabetic rats. **Conclusion:** From this study, it is concluded that the DADS a principle compound of garlic, definitely has the hypoglycaemic and hypolipidemic effect in diabetic rats, which is reducing the morbidity in diabetic cases without the adverse effects.

Keywords: Diallyl Disulphide (DADS), dyslipidemia.

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INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic syndrome affecting nutrient metabolism in general and glucose metabolism in particular. DM is characterised by persistent hyperglycemia and disturbance in the metabolism of carbohydrate, protein and fat associated with an absolute or relative deficiency in insulin secretion and action [1].

Diabetes mellitus may lead to many complications, which include diabetic ketoacidosis, hyperosmolar hyperglycaemic state, coronary heart disorder, dyslipidemia, retinopathy, nephropathy and neuropathy [2]. Hyperglycemia and dyslipidemia are chronic complications associated with diabetes that results from insulin deficiency. A similar picture can be seen in alloxan diabetic rats as it is known that alloxan induces profound beta-cell damage of islets of

Langerhans, leading to insulin deficiency. Umesh CSY *et al* have demonstrated that in alloxan diabetic rat, the lipid levels in plasma rise by 48-55% [3].

Most diabetic patients initiate their treatment with dietary restrictions and exercise and remain unsuccessful in controlling diabetes through lifestyle changes alone, prompting the need for therapeutic management. Drugs such as biguanides, sulfonylureas, thiazolidines, statins are some of the first-line therapeutic agents used in the management of diabetes. These drugs have both beneficial as well as adverse side effects [4]. Prolonged use of these drugs is associated with undesirable side effects which outweigh the benefits of the drugs.

To minimise the undesirable side effects of these drugs, many medicinal plants were used in the past, which have hypoglycemic and hypolipidemic activities. Studies conducted by the World Health Organisation (WHO) reported that 80% of the world's population relies on medicinal plants for their primary health care needs [5].

One of such medicinal plant, Garlic (*Allium sativum* Linn) is known for its anti-hyperglycemic, anti-hyperlipidemic, anti-atherogenic properties [6]. Many of these properties were attributed to the principle sulphur compound of garlic: Diallyl Disulphide (DADS) [7]. However, some of the published reports failed in demonstrating the hypolipidemic effect of garlic [8]. This discrepancy may be attributed to procedural/methodological shortcomings, such as inappropriate methods of randomisation, lack of dietary run-in period, short duration, inappropriate modes of administration and low statistical power [9]. The present study was undertaken, to address these discrepancies and to determine the hypoglycaemic and hypolipidemic effect of Diallyl Disulphide in alloxan-induced diabetic rats.

MATERIALS AND METHODS

Alloxan and Diallyl disulphide (DADS) was procured from Sigma Aldrich chemicals. All the other chemicals employed were of analytical grade.

Healthy Wister strain male albino rats weighing around 100-150 grams 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 bodyweight 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.

Induction of diabetes

Induction of diabetes was done by intraperitoneal injection of freshly prepared aqueous alloxan monohydrate (150mg per kg body weight) in sterile water to overnight fasted rats. 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.

On the completion of the stipulated period, rats were anaesthetised and sacrificed. Blood was collected in heparinised tubes.

Blood samples were employed for the estimation of blood glucose, insulin, total cholesterol, triacylglycerols, HDL cholesterol, VLDL cholesterol and LDL cholesterol.

STATISTICS

All the results are expressed as mean \pm 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 of less than 0.005 was selected as the point of minimal statistical significance.

RESULTS

The tables I and II show the effect of DADS on alloxan-induced diabetic rats and non-diabetic rats as gravimetry, blood glucose & serum lipid profile levels.

A significant loss of body weight was observed in group II rats as compared to group I rats, indicating a decrease in body weight in alloxan-induced diabetic rats. In group I and II, no significant change in final body weight was observed. In group IV rats, an improvement in final body weight was observed when compared to group II, suggesting DADS treatment improved the diabetic weight loss.

Table -1: Gravimetry: Changes in body weight in Normal and Alloxan Induced Diabetic rats before and after treatment with DADS (100mg/kg body weight) for 30 days.

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 \pm 9 ^a	257 \pm 16.8 ^a	251.5 \pm 6.9 ^a	253.5 \pm 9.3 ^a	0.7444	0.5388
2	Final Body weight (g)	276.6 \pm 11 ^a	238 \pm 18.1 ^b	266.6 \pm 8.1 ^a	242 \pm 7 ^b	15.38	0.0000
3	% Body weight Change	6 \pm 1.34 ^a	-8.23 \pm 2 ^b	5.6 \pm 0.9 ^a	-4.7 \pm 1.8 ^c	120.3	0.0000

Note: Each value is mean \pm 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).

Blood glucose, lipid profile is elevated in group II as compared to group I indicating insulin

deficiency or insufficiency in alloxan-induced diabetic rats. DADS has no significant effect on normal rats,

which is evident from the comparison of group III with group I. A significant hyperglycaemic and hypolipidemic effect of DADS on alloxan-induced

diabetic rats (group IV) was observed when compared to group II.

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

S.No	Parameter	Group I (n=6)	Group II (n=6)	Group III (n=6)	Group IV (n=6)	F Value	P Value
1	Blood Glucose (mg/dl)	99+7.2 ^a	473+24.2 ^b	93+6.0 ^c	323+25.8 ^d	580.1	<0.0001
2	Insulin (μU/ml)	15.8+1.7 ^a	7.9+0.9 ^b	14.9+1.6 ^c	11.8+1.5 ^d	34.13	<0.0001
3	Triacylglycerols(mg/dl)	60+4.0 ^a	90+9.1 ^b	49.8+6.3 ^c	64+7.4 ^d	33.09	<0.0001
4	Total Cholesterol(mg/dl)	58+4.5 ^a	100+6.1 ^b	53+4.8 ^a	77+5.41 ^c	89.33	<0.0001
5	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
6	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
7	VLDLCholesterol(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

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).

DISCUSSION

Alloxan induced insulin deficiency might be responsible for excessive burning of fat and loss of muscles, causing a decrease in per cent body weight in group II rats. DADS supplementation had a beneficial effect in reducing the loss of body weight in group IV rats.

Alloxan, specifically damaging the beta cell of Langerhans, causes a severe lack in insulin levels inducing a steep rise in plasma glucose levels. Elevation in blood glucose levels, as seen in the present study is in agreement with previous reports [10]. It is seen in DADS treated alloxan diabetic rats (group IV), the levels of plasma glucose are significantly lowered as

compared to group II rats. DADS can function as an active hypoglycemic agent probably by decreasing cellular NADH/NADPH levels, enhancing glucose utilising pathway. Insulin is a hypoglycemic hormone having alpha and beta chains interlinked by disulphide bridges. An NADPH dependent enzyme, insulinase is involved in insulin degradation. A decrease in NADPH levels caused by DADS may limit insulinase action causing an increase in the half-life of Insulin. This leads to prolonged insulin action and hypoglycaemia [11].

DADS is a disulphide and similar to any other disulphide it may undergo sulphhydryl exchange reaction with enzymes and proteins, as it is known that disulphides can undergo such a reaction [12].



Such a sulphhydryl exchange reaction with insulinase enzyme may delay insulin degradation and increasing its half-life, thus promoting hypoglycaemia. GLUT degrading systems or enzymes further degrade the glucose transporter molecules which are involved in glucose transport. A sulphhydryl exchange reaction by DADS with GLUT deteriorating systems may prolong the actions of GLUT molecules; hence more glucose is transported and utilised, thus favouring hypoglycaemia.

The anti-hyperlipidemic effect of garlic and its organosulfur compounds were previously established in hyperlipidemic rats [13]. However, few clinical trials failed to approve this lipid-lowering effect of garlic and its extracts, while many clinical trials validate this

effect [14]. DM induces dyslipidemia and gross alterations in plasma lipid levels. A significant increase is observed in plasma lipid profile in alloxan-induced diabetic rats (group II) as compared to normal rats (group I) is due to insulin deficiency caused by alloxan beta cell-damaging effect and agrees with earlier reports [15].

DADS (100 mg/kg body weight) significantly lowered the plasma lipids in diabetic rats (group IV) as compared to alloxan-induced diabetic control rats (group II), which is in concordance with other reports [16]. DADS is a disulphide that undergoes reduction to its thiols similar to any other disulphide by using NADPH/NADH as follows –



Diallyl Disulphide

It is proposed that such a reaction of DADS with NADPH may reduce cellular levels of NADPH, hence lowers fatty acid and cholesterol synthesis as

their synthesis requires sufficient supply of NADPH [17].

CONCLUSION

From the above findings, it may be concluded that 100 mg/kg body weight of DADS in alloxan diabetic rats has significantly lowered the elevated blood glucose and plasma lipids in alloxan diabetic rats. This hypoglycaemic and hypolipidemic effect of DADS in alloxan diabetic rats may be possible through lowering cellular NADH/NADPH levels which are required for the utilisation of glucose and biosynthesis of lipids. The reduction of DADS to its constituent thiols requires NADPH thereby lowering the cellular NADPH levels.

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