Indian Journal of Biochemistry & Biophysics
Vol. 57, October 2020, pp. 567-571

Effect of diallyl disulphide on hepatic glucose regulating enzymes in diabetic rats

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Received 19 July 2019; *revised 03 February 2020*

This study examines whether the glucose regulating enzymes mediate hypoglycaemic effect of diallyl disulphide (DADS) since the biochemical mechanisms by which the latter regulates hepatic glucose-metabolizing enzymes remain unknown. Hepatic hexokinase, glucose-6-P-D and pyruvate kinase are the important glucose metabolising enzymes that control blood glucose homeostasis and considered to be potential targets for antidiabetic drugs. DADS is an important phytoconstituent of garlic (*Allium sativum* Linn.) which has been reported to possess hypoglycaemic effect. Diabetes was induced in rats by alloxan and the diabetic rats were given DADS for 30 days and the effect was compared with the standard hypoglycaemic drug metformin. The levels of blood glucose and insulin were measured using spectrophotometer and by ELISA method respectively. Activities of hepatic hexokinase, glucose-6-PD, and pyruvate kinase enzymes in hepatic tissues were measured in DADS and metformin treated diabetic rats. DADS significantly reduced the level of blood glucose and simultaneously augmented those of insulin, pyruvate kinase, hexokinase and glucose-6-PD enzyme activities almost similar to metformin. The hypoglycaemic effect of this compound may be explained, in part, by its inhibition of these enzyme activities and improved hepatic glucose utilization. This observation offers scope for new therapeutic approach in treating diabetes particularly in insulin- resistant cases.

Keywords: Diallyl disulphide, Hexokinase, Pyruvate kinase, Glucose-6-phosphate dehydrogenase, Wistar rats

Diabetes mellitus (DM) is a metabolic syndrome characterised by hyperglycaemia with various pathological indicators often associated with abnormal lipid and glucose metabolism[1]. DM is caused by an insufficiency of either or both insulin secretion and action. Glucose is the required principle energy source for tissue cells and organs in humans[2]. However, chronic elevation of its levels results in diabetes and lead to secondary complications[3].

The liver plays a pivotal role in buffering the level of blood glucose by contributing either in hepatic glucose production (HGP) or utilization based on the prevailing plasma glucose level to maintain normal threshold value. The hepatocytes are coded with an array of regulatory mechanisms to maintain the process of glycogenolysis, glycolysis, glycogenesis, and gluconeogenesis[4]. There are several therapeutic options to maintain normoglycemia in diabetes individuals[5]. However, the most of the currently available antidiabetic medications such as biguanides, sulfonylureas, thiazolidinediones, and α-glucosidase inhibitors used for the treatment of type 2 diabetes[6] are also known to cause unwanted side effects and diminution after prolonged use[7]. Metformin is currently used as the drug of choice in the treatment of type 2 diabetes mellitus[8], being prescribed to at least 120 million people worldwide. As established in large prospective controlled clinical trials, metformin improves glycaemic control and reduces cardiovascular complications in diabetic patients and is also used to prevent chronic hypoglycemia[9]. Hence, search for newer drugs becomes necessary and the medicinal plants serve as promising source of drug discovery.

The traditionally used herbal products become the highly esteemed cradle of medicine[10] and also contribute as drugs of modern medicine. In this aspect, there are more than 65 species of plants with hypoglycaemic properties[11] as per the literature sources from numerous databases. One of the most dynamic plants is garlic (*Allium sativum* Linn.) that

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displayed the significant hypoglycaemic effect in earlier studies[12,13]. In addition, it is also reported to possess anti-hyperglycemic, anti-atherogenic, anti-hyperlipidemic properties and most of these activities were attributed to the principle compound DADS[14]. Ingestion of DADS decreases the risks of gastrointestinal cancers[15] and also have the capability to modify numerous biological mechanisms that might influence the favourable action on colon carcinogenesis and has been shown to have antiproliferative action on breast and liver carcinogenesis[15].

Most of the oral hypoglycaemic drugs currently used for treating diabetes possess antioxidant properties in addition to hypoglycaemic activity[16]. However, the available antidiabetic drugs often provoke undesirable side effects on prolonged use, and hence drugs with antioxidant potential without side effects might signify a useful ancillary pharmacological approach to the successful management of diabetes and its complications[17]. DADS offer a therapeutic action in oxidative stress-mediated changes caused by alloxan in the rat livers[18].

In this study, an effort has been made to assess the role of DADS on hepatic glucose utilization by regulating the glucose metabolising enzymes in diabetic rats and compare the action with metformin, a standard antidiabetic drug.

Materials and Methods

Chemicals

The chemicals such as DADS, metformin, and alloxan were bought from Sigma Aldrich Chemicals (St. Louis, U.S.A.) and other organic solvents and reagents used were of analytical grade procured from Thermo Fisher Scientific, India. The serum insulin ELISA kits were purchased from Enzo Life Sciences, Inc., and standards for pyruvate kinase, glucose-6-PD, hexokinase obtained from Randox Laboratories.

Animals

Male Wistar rats (175±25 g) used for the studies were kept in clean polypropylene cages and maintained under standard laboratory conditions ~~at~~ (22±2°C and 12 h alternating light-dark cycle). They had free access to standard pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*.

Experimental design

The rats were divided into four groups each comprising of six animals. Rats that received normal saline (1 mL/kg b.w., p.o.) served as normal control (Group 1). To 18 h fasted rats of other three groups, diabetes was induced by alloxan monohydrate (150 mg/kg; *i.p.*) dissolved in normal saline as a single dose. After 48 h of injection, hyperglycemia in rats was established by testing glucose positivity for 3 successive days. Rats with blood-glucose levels above 250 mg/dL were considered as diabetic and chosen for further studies[19]. Diabetic rats in group 2 remained untreated while to group 3 and 4 animals, DADS (100 mg/kg/day, p.o.) and metformin (150 mg/kg/day, p.o.) were administered respectively for 30 consecutive days.

After the experimental period, the animals were sacrificed via sodium pentabarbitone injection and blood was collected in heparinized plain tubes instantly for assays. Liver tissue was removed and washed with saline and tissue homogenate was prepared using PBS and maintained at −4°C until further assay. Blood plasma was separated by centrifugation (1700 × g, 15 min, 8°C) and immediately used for glucose measurement.

Assay of Insulin

Serum insulin level was measured by standard procedure using rat insulin ELISA kit[20]. During incubation, insulin in the sample reacts with peroxidase-conjugated anti-insulin anti-bodies and they bound to the plate well. The bound conjugated insulin was identified by reacting with 3, 3′, 5, 5′-tetramethylbenzidine. The reaction was stopped by adding acid to give a colorimetric end-point and optical density was measured with a microplate auto reader at a wavelength of 450 nm. The plasma glucose level was measured using the glucose diagnostic kit.

Assay of Hexokinase

The solution mixture contains 0.1 mL of 0.1 M ammonium phosphate buffer, pH 7.6, 0.1 mL of reduced diphosphopyridine nucleotide, 0.3 mL of 0.1% a-glycerophosphate dehydrogenase 0.1 mL of aldolase, 0.1 mL 0.4% adenosine triphosphate, 0.1 mL 0.07 M $MgCl_2$ and 0.1 mL of 0.1 M fructose-6-phosphate. To this mixture, 0.2mL of sample was added along with distilled water to get the final volume to 3 mL. Hexokinase activities were calculated as U/g tissue. Protein concentration in liver tissue was measured by Lowry method[21].

Assay of pyruvate kinase

Pyruvate kinase (PK) activity was determined in the liver homogenate of all the experimental groups. To 0.1 mL of sample added 100 µL of 50 mM of glycyl

glycine, 100 µL of 15 mM ethylene diamine tetra acetic acid (EDTA) and 100 µL of 5 mM potassium phosphate. The total activity of PK was resolute in the supernatants and measured in Units/g tissue[22].

Assay of Glucose-6-Phosphate dehydrogenase

The liver tissue (50 mg) homogenized in 200 µL assay buffer and centrifuged (13000 × g, 10 min.). The final volume of test sample was adjusted to 50 µL/ well with assay buffer in a 96-well plate. About 92 µL assay buffer, 8 µL GDH developer, and 10 µL 2 M glucose were added to each well-comprising test samples. The mix was incubated for 3 min at 37°C and OD was measured at 450 nm in a microplate reader[23].

Statistical analysis

Data obtained from the experiments are presented as the mean ± SD of six values. For statistical analysis, data were subjected to one-way analysis of variance (ANOVA) followed by Student's *t*-test. A level of P <0.05, P <0.01, P <0.001 was taken as significant. The statistical analysis was done using the SPSS statistical package (Version 22.0).

Results

Effect of DADS on glucose and insulin levels

The effect of DADS on the levels of blood glucose and plasma insulin in alloxan- induced diabetic rats are presented in (Table 1). Diabetic rats showed significantly (P <0.001) elevated fasting blood glucose levels and decreased insulin levels when compared to control rats. The altered levels of insulin and blood glucose were reverted to near normal values after oral treatment with DADS suggesting the regulation of glucose homeostasis. The normalising level of insulin in the DADS fed group reveals the hypoglycaemic action which is comparable with that of metformin activity.

Table 1— Effect of DADS and metformin on plasma glucose and insulin levels in diabetic rats.

Groups	Glucose (mg/dL)	Insulin (pg/mL)
Normal control	85.76 ±1.33	0.36 ± 0.00
Diabetic rats	286.70 ± 1.20***	0.15 ± 0.00***
Diabetic + DADS	99.46 ± 0.32^{c}	0.27 ± 0.00^{c}
Diabetic + Metformin	108.80 ± 0.74$^{@@@}$	0.23 ± 0.00$^{@@@}$

Results are expressed as Mean ± SD (n =6); ***P <0.001 as compared to normal control group; $^{@@@,c}P$ <0.001 as compared to DM control group.

Effect of DADS on glucose metabolising enzymes

The results indicated the role of DADS in influencing hepatic glucose utilization in alloxan diabetic liver by increasing the activities of glucose metabolising enzyme hexokinase and regulatory enzymes pyruvate kinase, and G-6PD are presented in (Figs. 1-3). It is evident from the data that activities of pyruvate kinase, hexokinase and glucose-6-PD were significantly (P <0.01) lowered in the diabetic group when compared to normal rats whereas, the enzyme activities are significantly elevated in DADS and metformin- treated rats as compared to diabetic rats. DADS influence increased glucose utilization by increasing glycolytic enzyme activity which is comparable with standard anti-diabetic drug metformin.

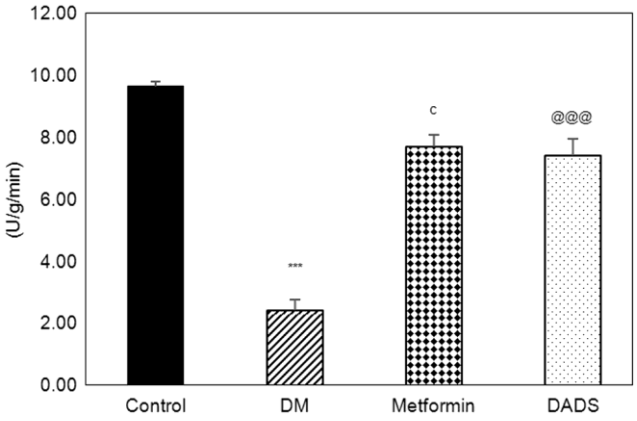

Fig. 1 — Effect of DADS and metformin on hepatic Hexokinase activities in diabetes mellitus induced rats. Results are expressed as Mean ± SD (n =6); ***P <0.001 as compared to normal control group; ^{c}P <0.001 as compared to DM control group; $^{@@@}P$ <0.001 as compared to DM control group

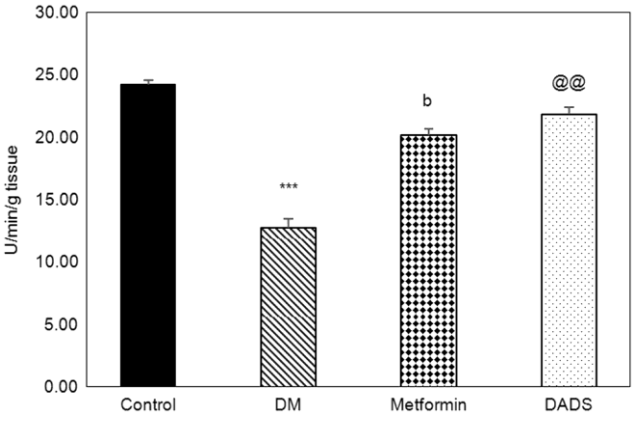

Fig. 2 — Effect of DADS and metformin on hepatic Pyruvate kinase activities in diabetes mellitus induced rats. Results are expressed as Mean ± SD (n =6); ***P <0.001 as compared to normal control group; ^{b}P <0.01 as compared to DM control group; $^{@@}P$ <0.01 as compared to DM control group

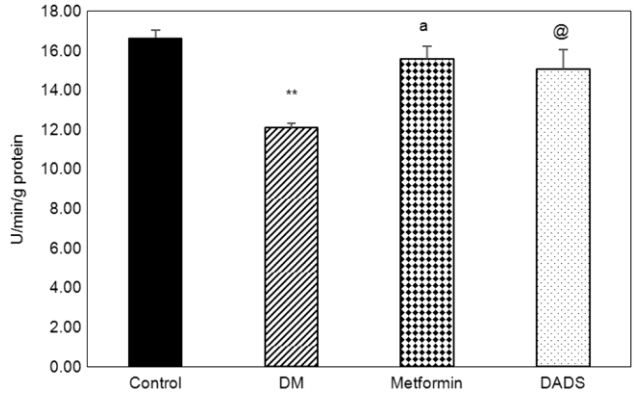

Fig. 3 — Effect of DADS and metformin on hepatic Glucose-6-phosphate dehydrogenase activities in diabetes mellitus induced rats. Results are expressed as Mean ± SD (n =6); $^{**}P$ <0.01 as compared to normal control group; ^{a}P <0.05 as compared to DM control group; $^{@}P$ <0.05 as compared to DM control group

Discussion

The major goal of diabetes management is to keep the level of blood glucose in the normal range by proper utilisation of cells. Blood glucose control is an important constituent in preventing acute or long-term diabetic complications[24]. Though insulin resistance is the starting pathogenic factor in type 2 diabetes, β-cell failure is responsible for insulin deficiency and impaired glucose tolerance to explicit type 2 diabetes[25]. Insulin resistance in the peripheral tissues allied with increased hepatic glucose production to release into blood stream are responsible for increased fasting blood glucose levels in diabetic rats.

DADS treatment for 30 days reduced the level of blood glucose in alloxan-induced diabetic rats, a desirable criterion for any potential anti-diabetic agent and also no hypoglycaemic condition was observed in treated group[26]. DADS treatment also significantly increased the low serum insulin and induced attenuation of glucose metabolic disorder through regulations of glucose metabolic enzymes.

Diabetes induced hyperglycaemia is due to lack of insulin or subnormal functioning of insulin arises because of disturbances in glucose metabolism caused by a reduced activity of several regulatory key enzymes of glycolysis such as HK, PK and G-6PD, resulting in impairment in glucose utilization by peripheral cells and augmented hepatic glucose production. In diabetic condition, the activities of these enzymes are hindered due to insulin resistance because it is an insulin-dependent enzyme[27]. Markedly reduced level of insulin diabetic rats eventually leads to the impairment in the effect of hexokinase since insulin deficiency which finally leads to the onset of chronic hyperglycemia which is a hallmark of diabetes[28].

The activities of key enzymes of carbohydrate metabolism such as hexokinase, pyruvate kinase, and glucose-6-phosphate dehydrogenase in hepatic tissues were evaluated to establish the regulatory effect of DADS in glucose homeostasis in comparison with the hypoglycaemic drug Metformin. An increase in glycolytic enzymes (HK and PK) enhances peripheral glucose utilization through glycolysis and could contribute to the anti-hyperglycaemic effect of DADS. The hypoglycaemic action of DADS is highly operative in bringing down the level of blood glucose to near normoglycemia and also increased glucose utilization through glycolysis by increasing the activities of key enzymes. This may be due to its ability to inhibit CYP2E1, enhance antioxidant enzyme activities, and suppress inflammation[29].

Goudappala *et al* and Yusuf *et al* have reported that hypoglycemic effects of the antidiabetic drug are due to the mechanism arbitrated by hepatic glucose-regulating enzymes[29,30]. Upon oral treatment with DADS, the diabetic rats displayed not only the elevated hepatic hexokinase, pyruvate kinase, and G-6-PDactivity but also significantly lowered the insulin levels compared with the standard metformin group, which might explain the glucose-lowering activity of DADS.

Conclusion

The results of the study revealed how the regulation of glucose metabolising enzymes could be utilised as targets in treating type 2 diabetes either by directly using targeted inhibition or indirectly as a consequence of inducing energy stress. The later mechanism may contribute significantly to the apparent glucose-lowering effect of many biologically active secondary metabolites. The increased activity of these glucose regulating enzymes by DADS facilitates glucose oxidation leading to controlled glucose homeostasis in diabetic conditions. However, further studies are warranted at molecular levels to elucidate the actual mechanism of action of DADS by which hypoglycaemic effect is brought about.

Acknowledgement

The authors are indebted to the authorities of Saveetha Institute of Medical and Technical Sciences, Chennai and Subbaiah Institute of Medical Sciences, Shivamogga for the encouragement, facilities, and support.

Conflicts of interest

All authors declare no conflict of interest.

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