IJPSR (2022), Volume 13, Issue 6 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 15 September 2021; received in revised form, 07 February 2022; accepted, 02 May 2022; published 01 June 2022

EFFECT OF DIALLYL DISULFIDE (DADS) ON GLUCOSE UTILIZATION IN DIABETIC ERYTHROCYTES

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Keywords:

Diabetes - Diallyl disulphide - Erythrocyte -Garlic - Glucose utilization

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ABSTRACT: Background & objectives: Diallyl disulphide (DADS) is an active component of garlic that has been claimed to have hypoglycemic effect and are beneficial in achieving glycemic control in diabetes mellitus (DM). The present study was conducted to assess the glucose utilization in erythrocytes in diabetic subjects after the exposure of DADS. **Methods:** The blood samples were collected from diabetic subjects; the plasma and erythrocytes were separated. The fasting plasma glucose levels were measured and the separated erythrocytes from diabetic blood samples were preincubated and posted incubated with DADS. The glucose utilized by erythrocytes and the percentage of glucose utilization were measured. **Results:** The present study results showed that diabetic erythrocytes exposed to DADS showed a significant rise of glucose utilization compared to untreated erythrocytes, which signifies the positive influence of DADS on glucose utilization in diabetic patients' erythrocytes. **Conclusion:** DADS, through the formation of thiol derivatives, promotes glucose utilization by mimicking the actions of insulin in diabetic erythrocytes after post-incubation. This study can be concluded that DADS can be used as a therapeutic drug to improve glucose utilization in diabetic patients.

INTRODUCTION: Diabetes Mellitus is a chronic metabolic disorder principally involving intermediary metabolism in general and glucose metabolism in particular due to subnormal functioning of insulin. The global prevalence of this disease is about 150 million and according to World Health Organization (WHO) and the expected number of people suffers from this disease worldwide may double by 2025 [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.13(6).2492-96

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.13(6).2492-96

Glucose is the major energy source for the red blood cell; the mature erythrocytes, however, lack the oxidative enzymes present in the mitochondria of most other cells. The red blood cells cannot depend on aerobic glycolysis to extract energy from glucose as in Kreb's cycle. They, therefore, use the Embden-Meyerhof pathway to aerobic process glucose into usable energy or adenosine triphosphate (ATP).

The red blood cells can limit aerobic glycolysis through the pentose phosphate shunt or the hexose monophosphate shunt. However, the major role of the hexose monophosphate shunt is not the oxidative metabolism of glucose but rather the generation of reduced NADPH [2]. Apart from insulin and other hypoglycemic agents, many herbal preparations have been tried to improve

glucose utilization in Diabetes mellitus. The World Health Organization (WHO) has also recommended the evaluation of plants effectiveness in controlling the spreading of diabetes mellitus as we lack in safe modern drugs [2]. Many medicinal plants when subjected to scientific experimentations have yielded positive reports which the contemporary system of medicine could take up. Among the various biological activities of the medicinal plants studied, the hypoglycemic and hypolipidemic actions have been the most common ones [3]. Since ancient times, these herbs and their extracts have been employed to control hyperglycemia of diabetes mellitus [4].

Previous studies have shown that allium group of plants, specifically garlic (*Allium sativum* Linn) has shown significant hypoglycemic effect in diabetes mellitus which is attributed to their sulfur compound, specifically diallyl disulphide (DADS). Though it is known that garlic or one of its active principles, DADS as well as other disulphides may influence insulin action or increase insulin's half-life, the actual mechanism of action is still obscure [5]. Therefore, a study was designed to prove the influence of DADS on glucose utilization in diabetic erythrocytes.

MATERIALS AND METHODS:

Chemicals & Drugs: Diallyl disulfide (DADS) used in the present study were procured from Sigma-Aldrich Chemicals (St Louis USA).

Sample Collection: The samples were collected from type 2 diabetic subjects attending the medical OPD of Subbaiah Institute of Medical Sciences, Shimoga, Karnataka, India, in the age group 30-55 years. Fasting heparinized blood sample (6-8 ml) was collected from these subjects (both normal as well as diabetic subjects) after obtaining informed consent. The normal blood was taken from the employees of Subbaiah Institute of Medical Sciences and other affiliated hospitals, Shimoga, India.

Exclusion Criteria: Diabetes subjects below 30 years and above 60 years were excluded. Diabetes associated with various complications like Nephropathy, Cardiopathy, and Retinopathy were excluded. Postmenopause diabetic females, diabetic subjects with non-pancreatic endocrine disorders, neurological disturbances, and patients receiving hormone therapy other than insulin were excluded.

Separation of Erythrocytes: The blood samples were centrifuged to separate the plasma and erythrocytes. The plasma samples were used for the estimation of glucose [6]. The erythrocytes are washed three times with a 3ml aliquot of normal saline. The washed erythrocytes were suspended in an equal volume of normal saline to give a 50% saturated erythrocyte suspension and were employed for glucose utilization studies.

Experimental Design: The study included the diabetic erythrocytes preincubated and posted incubated with DADS, and the glucose levels are measured.

Glucose Utilization Studies: The glucose contents of fasting plasma glucose, preincubated and post incubated erythrocyte samples were estimated. Glucose utilization in normal erythrocytes, diabetic erythrocytes, and DAD Sex posed diabetic erythrocytes were studied. The normal erythrocyte suspension (1ml) was taken in one tube, and 1 ml of diabetic erythrocyte suspensions were taken in two different incubation tubes. 1 ml of freshly prepared glucose solution was added to all tubes and mixed well. DADS (10µl) were added to one tube containing diabetic erythrocyte suspension and mixed well. Immediately 0.5ml from all tubes were pipetted out into three tubes containing 4.5ml of 10% TCA.

The solutions were allowed to stand at room temperature for 15 min. The contents of the tubes were thoroughly homogenized using a Remi homogenizer and later centrifuged at 3000rpm for 5 minutes; the clear supernatant obtained was used for glucose estimation[6]. The value obtained was considered a pre-incubation glucose level. The residues were incubated with DADS at 37 °C in a thermostatically regulated water bath for 60 minutes. After that, 0.5 ml of the solution was separately pipetted out into three different tubes containing 4.5ml of 10% TCA. The tubes were kept at room temperature for 15 minutes for protein precipitation. After 15 min, all the tubes were again homogenized and centrifuged at 3000rpm for 5 min. The values obtained were considered as post incubation glucose levels.

Statistical Analysis: Results were measured as mean ± S.E.M, and the statistical significance was calculated using a student t-test.

Statistical difference between the means of independent groups was analyzed using a one-way analysis of variance (ANOVA). P values less than 0.05 were considered significant.

RESULTS: Fasting plasma glucose levels were significantly elevated ($p<0.001$) in the diabetic erythrocyte group compared to the normal erythrocyte group and DADS exposed diabetic erythrocyte group non-significant changes in fasting glucose levels **Fig. 1**. The DADS post incubated erythrocytes showed a decrease in glucose level as compared to diabetic erythrocytes whereas non-significant changes were noted in preincubated erythrocytes. A significant decline ($p< 0.001$) was seen in the percentage of glucose utilized by diabetic erythrocytes when compared to normal erythrocytes. A significant elevation ($p< 0.001$) was observed in the amount of glucose utilized by erythrocyte as well as the percentage of glucose utilization by erythrocytes in DADS exposed diabetic erythrocytes compared to diabetic erythrocytes untreated, suggesting the DADS influence on glucose utilization in diabetic erythrocytes **Fig. 2**. Therefore, the present study showed that glucose utilization is significantly elevated in DADS-exposed diabetic erythrocytes, further establishing the positive influence of DADS on glucose utilization in diabetic erythrocytes.

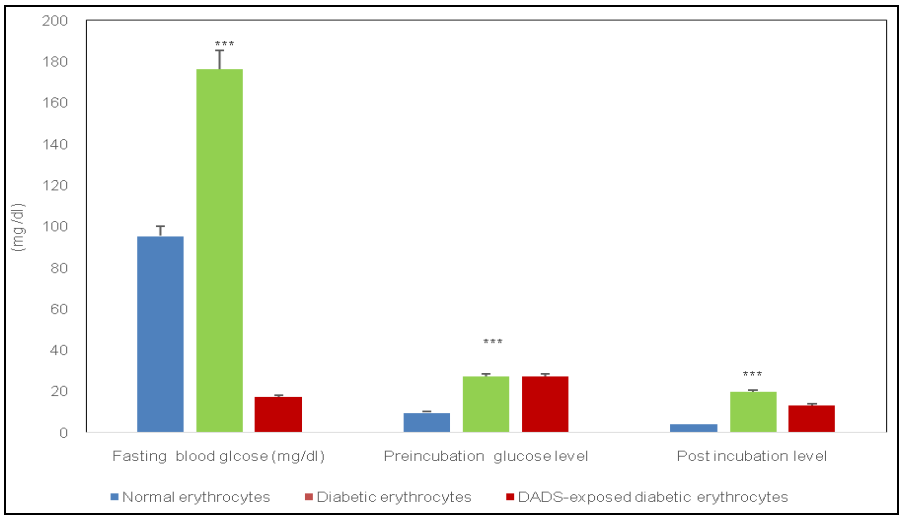

FIG. 1: EFFECT OF DADS ON GLUCOSE LEVELS IN DIABETIC ERYTHROCYTES. The values are expressed as mean ± SEM; ***$p<0.001$ statistically significant as compared to the normal erythrocyte.

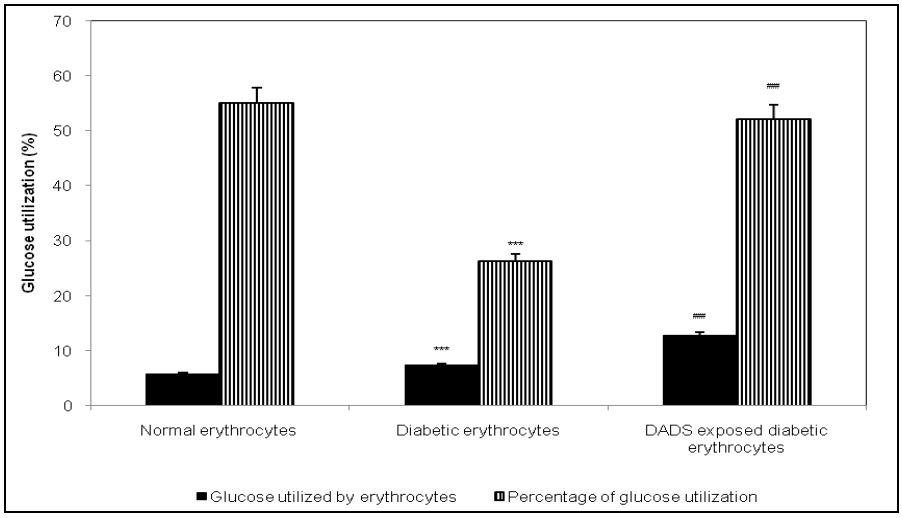

FIG. 2: PERCENTAGE OF GLUCOSE UTILIZED IN DADS TREATED DIABETIC ERYTHROCYTES. The values are expressed as Mean ± SEM; ***$p<0.001$ statistically significant as compared to the normal erythrocyte.###$p<0.001$ statistically significant as compared to the diabetic erythrocyte.

DISCUSSION: Diallyl disulphide is an aliphatic low molecular weight disulphide that undergoes degradation to produce thiols in the presence of NADPH/GSH dependent reductase [8]. The thiols produced may alter the cellular thiol-disulfide ratio, which is detrimental to the functioning of thiol enzymes involved in glucose metabolism. It is known that low molecular weight thiols influence certain glycolytic enzymes' activity, thus favoring glucose utilization [9, 10]. The present study results concerning glucose utilization by diabetic erythrocytes exposed to DADS showed a significant rise compared to diabetic erythrocytes. This is consistent with the above statement and shows the glucose utilization promotional-effect DADS in diabetic patients' erythrocytes. The allyl thiols, produced by the degradation of DADS have low molecular weight and have influenced glycolytic enzymes like hexokinase, phosphofructokinase and pyruvate kinase [11].

Similar to any other disulphide, DADS undergo sulphydryl exchange reaction with cellular thiol proteins or thiol enzymes [12]. DADS also undergo a similar sulfhydryl exchange reaction with Protein Tyrosine Phosphatase (PTP) and inhibit their activity. Therefore, PTP's and other insulin receptor substrates (IRS) may remain functioning, thereby enhancing glucose uptake and utilization. Earlier studies [13, 14] have reported that certain disulfides when given orally or intraperitoneally, may decrease certain glycolytic pathway enzymes. This can be explained that DADS might have undergone sufficient reduction to produce its thiol derivatives, and these thiols might have interacted with sulphydryl enzymes of the glycolytic pathway, thus favoring their actions by keeping these enzymes in their thiol forms.

Studies indicate that the active principles of onion [14] (dipropyl disulfide) and garlic [15] (diallyl disulfide) are hypoglycemic and increase glucose utilization. Many enzymes of carbohydrate metabolism (specifically glycolytic pathways including hexokinase, phosphofructokinase, and pyruvate kinase) are thiols and are expected to be altered by cellular thiol concentrations [16]. The data shown in the present study is in agreement with the hypothesis that DADS exposed to diabetic erythrocytes might have improved the cellular thiol levels hence keeping the enzymes in their thiol nature which favours the activities in increased glucose utilization. It has been observed that certain low molecular weight thiols mimic the actions of insulin possibly by acting as substrates for the NADPH oxidase (NOX) system [17]. Thus, it may show certain effects of insulin, hence favour glucose utilization. The action of low molecular weight thiols through the NOX system may increase cellular NADP levels and facilitate glucose utilization through the HMP pathway. The results showed in **Fig. 1** and **Fig. 2** a significant ($p<0.001$) rise in glucose utilization in DADS-exposed-diabetic erythrocytes as compared to diabetic erythrocytes. DADS, a low molecular weight disulfide of garlic, exhibited glucoseutilization-promotional effects in DADS-exposed-diabetic erythrocytes.

CONCLUSION: DADS might have undergone sulphydryl cleavage to produce its thiol counterparts, and these thiols might have mimicked similar DADS actions. Hence, there is a glucose-utilization-promotional effect in DADS-exposed diabetic erythrocytes. Thus, through the formation of thiol derivatives, DADS promotes glucose utilization of DADS-exposed diabetic erythrocytes by mimicking the actions of insulin. This study can be concluded that DADS influences glucose utilization in diabetic erythrocytes. DADS can be used as a therapeutic adjuvant to improve glucose utilization in diabetic patients. Further study is needed with many numbers of subjects to prove DADS clinical utility as a hypoglycemic substance.

ACKNOWLEDGEMENT: This study was supported by the Indian Council of Medical Research Short Term Studentship Program 2018 (ICMR-STS 2018).

Funding: The research funding was awarded by Indian Council of Medical Research Short Term Studentship Program 2018 (ICMR-STS 2018).

CONFLICTS OF INTEREST: Nil

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How to cite this article:
Rao A, Nagendra A, Gowda CVY, Kashinath RT and Goudappala P: Effect of diallyl disulfide (dads) on glucose utilization in diabetic erythrocytes. Int J Pharm Sci & Res 2022; 13(6): 2492-96. doi: 10.13040/IJPSR.0975-8232.13(6).2492-96.