ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: https://ijrps.com

Influence of Diallyl disulphide on Hepatic Gluconeogenesis suppression by CREB Binding protein phosphorylation

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Article History:

Received on: 12.10.2018
Revised on: 25.03.2019
Accepted on: 28.03.2019

Keywords:

Diabetes,
Diallyl disulphide,
Hepatic glucose,
CREB,
Alloxan

ABSTRACT

Diabetes is an important human ailment affecting many lives in different countries. Diallyl disulfide (DADS), the antidiabetic compound found in garlic, acts as a therapeutic agent in diabetes mellitus condition. This research aimed to investigate the role of DADS on the gluconeogenic mechanism in the liver tissue and the potential involvement of CREB in glucose homeostasis in a Wistar rat model. The alteration in the body weight, liver weight and glycogen content in diabetic rats were prevented by this therapeutic compound: the cAMP-responsive element-binding protein (CREB), an important transcriptional regulator of the gluconeogenic mechanism. The glucose uptake potential was studied by the expression of CREB protein in DADS treated diabetic rats using the western blotting technique. A high level of hepatic CREB protein expression was noted in diabetic status in the chronic hyperglycemic model which was reversed by DADS. The antihyperglycemic effect of DADS was almost similar to that of known antidiabetic drug metformin. The therapeutic action of DADS on diabetic status is due to the control of the glycemic imbalance in liver tissue.

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ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v10i2.536

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IJRPS | https://ijrps.com

INTRODUCTION

According to world health organization (WHO) the diabetes prevalence is expected to increase by 35%. At present, about 150 million peoples had diabetes worldwide and this number could be increased up to 300 million or more by the year 2025 (Ramachandran and Sneha latha, 2002). In the urban areas of India diabetes is one of the major health problem. The liver regulates glucose metabolism, at fasting condition it produces glucose by increasing glycogen breakdown and at postprandial condition it stores glucose as glycogen. Several anabolic and catabolic fluxes have been encompassing in glucose metabolism of hepatic tissue that have distinct modes regulatory mechanisms such as hepatocyte-autonomous (direct) and hepatocyte-non-autonomous (indirect) (Ekberg *et al.,* 1999). The total hepatic glucose formed from gluconeogenesis, glycogen synthesis, glycogenolysis, glycolysis and other pathways.

During fasting condition in human beings liver sends the glucose to maintain glucose homeostasis and fuel to red blood cells, renal medullary cells and neurons. The liver helps in the disposal of extra glucose overloads by increasing glycogenesis process and by suppressing hepatic glucose output

(Moore *et al.,* 2012). Acute regulation of liver glucose homeostasis is regulated through alteration in protein phosphorylation, substrate availability, allostery and redox state. The cAMP-responsive element binding protein (CREB), a potent activator of the gluconeogenic regulation program in hepatic tissue, may act as a vital therapeutic target to reduce glucose output by the liver (Everette *et al.,* 2013). The hepatic hyperglycemic status can be regulated by inhibiting the enzyme glucose-6-phosphatase and inhibition of CREB protein.

Though there are various clinical approaches tried to cure diabetes, and its secondary complications cause normal cell dysfunction. Now a day's herbal formulations are of major interest to human society due to lesser side effects and low cost. Diallyl disulphide (DADS or 4, 5-dithia-1, 7-octadiene), an important phytocompound from garlic possess antitumor and immunomodulatory functions have attracted the attention of researchers (Omar *et al.,* 2010). Chinese leek oil, chive oil and garlic oil are the natural sources of DADS compound. Researchers found that DADS is a useful therapeutic antiinflammatory and antibacterial compound (Park *et al.,* 2012). Previously reported that DADS have antioxidant and antiulcer activities (Xie *et al.,* 2018; Lee *et al.,* 2015). And also DADS influence the insulin action in alloxan induced diabetic animals (Kumari *et al.,* 1995). Earlier reports support the current study to elucidate the effects of DADS on diabetes mellitus for the extensive development and use of garlic extracts.

The research evidence about the role of DADS on the gluconeogenic mechanism of liver tissue has not been carried out. Therefore, this study was aimed to understand the effect of DADS on 'gluconeogenic capacity' of the liver in alloxan induced rat model.

METHODOLOGY

Chemicals and their sources

The chemicals such as DADS, metformin and alloxan were obtained from Sigma Aldrich Chemicals (St. Louis, U.S.A.) and other organic solvents and chemicals used for this study were of analytical grade procured from Thermo fisher Scientific, India.

Animals

The male albino rats of wistar strain (150 - 200 g) were used for the present studies. Animals were housed in clean polypropylene cages and maintained under standard laboratory condition at temperature 22±2°C and 12 h alternating light - dark cycle. The rats were given RO purified water for drinking and standard pellet diet purchased from Hindustan Lever Limited. The experiments were conducted as per the guidelines of CPCSEA under the supervision of Institutional Animal Ethics Committee. Ethical clearance was given from IAEC of Basveshwara Medical College and Hospital (BMCH), Chitradurga (IAEC NO: BMCH/IAEC/04 Biochem/2015).

Experimental design

For the experimental work, the animals were segregated into 4 groups comprising of 6 animals in each group. The rats received normal saline (1ml/kgb.wt) orally were served as normal control; For another three sets of rats diabetes was induced by using the chemical Alloxan monohydrate (150 mg/kg; i.p.) dissolved in normal saline to 18 h fasted rats as a single dose. After 48h injection, hyperglycemia in rats was confirmed by testing glucose positive for 3 consecutive days. Rats with blood-glucose levels above 250 mg/dL were considered as diabetic and chosen for further experimental studies (Chougale *et al.,* 2007). Diabetic rats treated with metformin (150mg/kg/day) orally as a single dose for 30 days served as a standard control. To analyse the therapeutic role of DADS, diabetic rats treated with diallyl disulphide (100mg/kg/day) orally as a single dose for 30 days (Prashanth kumar *et al.,* 2018).

On day 1 (Initial) and day 30 (Final), animals were weighed using animal weighing balance, and body weights were noted. After 30 days, the animals were not provided food and water for overnight. The rats were euthanized under chloroform vapor. The liver was dissected out, washed to remove all the blood from the tissues using ice-cold physiological saline and weighed. The homogenate of liver tissue was prepared by using PBS and maintained at -4ºC until further assay. The tissue homogenate was used for the estimation of glycogen content using commercially available diagnostics kits (SPAN diagnostics) and CREB protein expression.

Assay of CREB protein expression by western blotting

The protein samples (30 µg protein) isolated from liver tissues were loaded onto 10% (w/v) sodium dodecyl sulphate (SDS)-polyacrylamide gel. After this process the gel were transferred electrophoretically to a nitrocellulose membrane. The membranes were washed with TBST buffer (10 mM Tris-base, 0.15 M NaCl, and 0.05% (w/v) Tween 20) for 10 min. The membrane was then blocked with 5% calf serum albumin. Next step the membrane was incubated with primary polyclonal anti-CREB antibody with a dilution of 1:3000 for 2

h. The membrane was then probed for 1 h at room temperature with horseradish peroxidise-labelled secondary antibody (anti-rabbit immunoglobulin G (IgG); 1:3000) for 5 h at room temperature. The CREB protein expression was detected in the membrane using 3,3-diaminobenzidine tetrahydrochloride (DAB) as the HRP substrate (Xie *et al.,* 2019). Finally, the western blots quantification was done by scanning the blots with Adobe Photoshop and density of the bands were measured by densitometry with Bio-1D Image.

Statistical analysis

The values obtained from these research results are expressed as mean±SD. For statistical comparison one-way analysis of variance (ANOVA) followed by post hoc Dunnett's T3 test was carried out. A level of $p<0.05$, $p<0.01$, $p<0.001$ was taken as significant.

RESULTS AND DISCUSSION

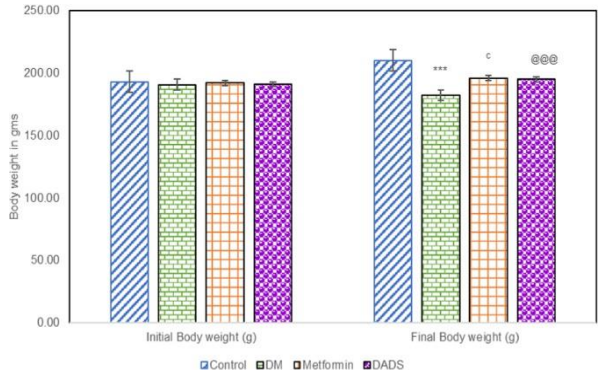

Figure 1: Effect of DADS and metformin on Initial and final body weight changes in diabetes mellitus induced rats. Results are expressed as Mean ± SD (n =6); ***P < 0.001 as compared to normal control group; ^{a}P< 0.05 as compared to DM control group; $^{@}$P < 0.05 as compared to DM control group

The initial and final body weight changes of the experimental animals were shown in the Fig 1. Therefore, the initial mean body weight decreased by about 8% as compared with final body weight in diabetic rats. There was a significant gain 8% in DADS treated and metformin treated diabetic rats as compared with untreated rats. The body weight gains between metformin and DADS treated rats was similar as compared and no significant changes were noted.

The complications of diabetes which include the abnormalities produced in lipids and proteins are the major etiologic factors. In diabetic control, there was an increase in liver weight and reduced glycogen content as compared to normal animals (Fig.2). Increased hepatic glucose production also contribute to the development of postprandial hyperglycemia, which is due to a reduced action of insulin to lower the HGP as well as impaired insulin-stimulated glucose uptake in liver tissues (Gastaldelli *et al.,* 2000).

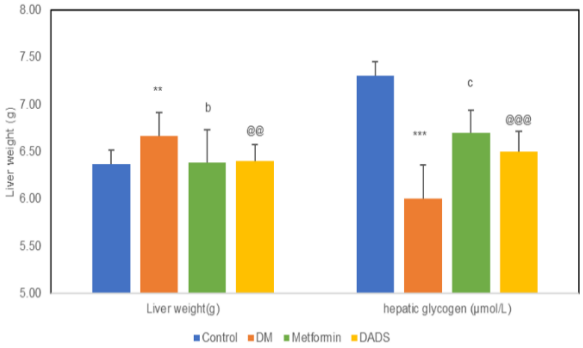

Figure 2: Effect of DADS and metformin on Liver weight changes and hepatic glycogen content in diabetes mellitus induced rats. Results are expressed as Mean ± SD (n =6); **P < 0.01 as compared to normal control group; ^{b}P< 0.01 as compared to DM control group; $^{@@}$P < 0.01 as compared to DM control group. ***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

We noted that the test compound DADS increased the hepatic glycogen level and lowered liver weight significantly similar to that of metformin. This may be attributed mainly due to the improved hepatic glycemic control mechanisms in diabetic rats. The previous experimental research conducted upon this work proved the glucose utilization promotional-effect of DADS in alloxan diabetic liver (Prashanth kumar *et al.,* 2018). The current reports about the action of DADS on hepatic glucose homeostasis are concomitant with the previous findings. The enhanced glucose utilization by DADS is through increasing glycolysis in the liver as well as its utilization through hexose monophosphate (HMP) pathway as suggested by increased hexokinase (HK) and glucose-6-phosphate dehydrogenase (G6PD) activities.

In hepatic gluconeogenic gene program, cAMP-responsive element-binding protein (CREB) is a vital positive transcriptional regulator protein (Erion *et al.,* 2009). The ability to induce gluconeogenesis in liver by CREB is dependent mainly on CREB coactivator transducer of regulated CREB activity. The CREB activates the gluconeogenic mechanism in liver, suggest that CREB used as a therapeutic target agent to reduce hepatic glucose production. The chronic hyperglycemia coupled with increased glucose output by liver characterized by increased gluconeogenesis, is the major aspect of type 2 diabetes. An effective *in-vivo* method to efficiently decrease the expression of CREB protein provides

a potential targeting therapy for hyperglycemia and type 2 diabetes treatment.

CREB protein expression levels were quantified in hepatic tissues using the western blotting technique (Fig.3). The protein expression was upregulated in diabetic rats compared to the normal control group. DADS treatment ameliorates the expression of CREB protein in diabetic rats significantly in comparison with untreated diabetic rats. There was no significant difference between metformin and DADS administered rats in the expression of this protein (Fig.4). The lowered glucose levels were observed when hepatic CREB expression levels were reduced by DADS in the current study which is concordant with the previous results (Erion *et al.,* 2009). Together, these results suggest CREB as a novel therapeutic target for lowering blood glucose levels in T2DM patients by inhibiting excess gluconeogenesis in the liver.

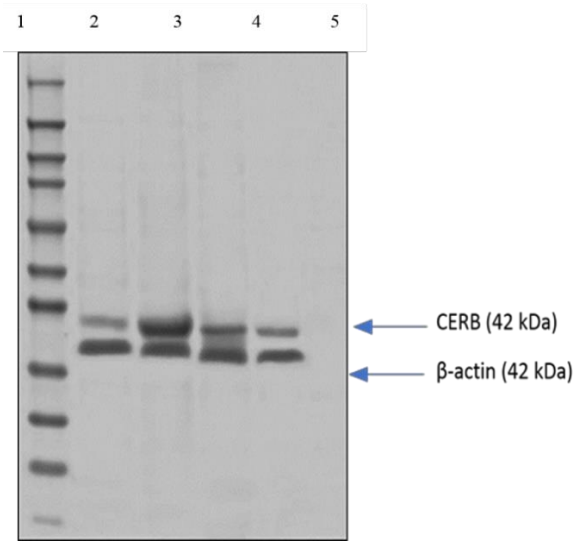

Figure 3: CREB protein expression by western blotting assay. Lane 1- Marker lane; Lane 2 – Normal control; Lane 3 – Diabetes induced; Lane 4 – Diabetes treated with Metformin; Lane 5 – Diabetes treated with DADS

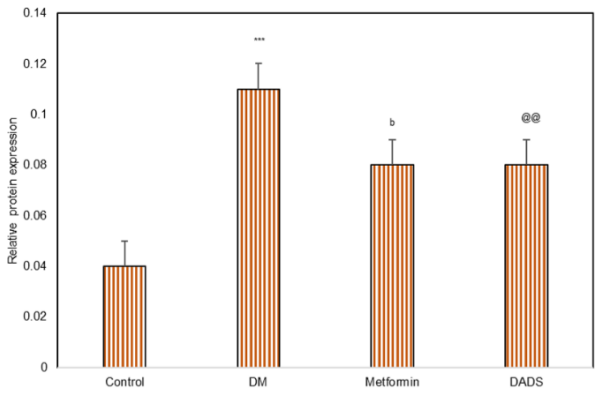

Figure 4: Quantitative data for CERB protein expression. The graph values are expressed in intensity units and represented as means ± SD (n = 6). Statistical analysis was done using one-way ANOVA with Dunnett's post hoc tests. ***p<0.001 statistically significant as compared to normal control. [b]p<0.01 and [@@]p<0.01 statistically significant as compared to diabetic control.

CONCLUSION

In the present study, we found that DADS may act a therapeutic compound on hyperglycemia in chronic diabetic condition and also acts as a hepatic gluconeogenesis regulator, decreased HGP and improved hepatic and peripheral insulin sensitivity. We can conclude that suppression of CREB protein expression by DADS may reduce the hepatic gluconeogenic capacity in T2DM patients and lowers the blood glucose level to normal status.

Acknowledgements

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

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