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Evaluation of plasma H_2S levels and H_2S synthesis in streptozotocin induced Type-2 diabetes-an experimental study based on *Swietenia macrophylla* seeds

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PEER REVIEW

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Comments

Over all the paper is very informative and gives very scientific information, which makes us to rethink about the relationship of H_2S and diabetes mellitus.
Details on Page S486

ABSTRACT

Objective: To evaluate the plasma H_2S levels and H_2S synthesis activity in streptozotocin induced type 2 diabetes rats compared to the healthy controls and also to observe the effect of the aqueous extract of *Swietenia macrophylla* (*S. macrophylla*) seeds on the experimental groups.
Methods: Seeds of *S. macrophylla* were separated, washed, shed-dried and finally extract was prepared. Thirty two wistar rats were selected for the experimental study. Streptozotocin was used for the induction of diabetes. H_2S concentration in plasma was measured. H_2S synthesizing activity in plasma was measured. Statistical analysis have done using Microsoft excel, Office 2003. Values were expressed by mean±SD. $P<0.05$ were considered statistically significant.
Results: Fasting blood glucose level (7.74±0.02) mmol/L was significantly increased in diabetic rats. The glucose levels are significantly lowered in the rats treated with metformin (5.48±0.03) mmol/L as well as with aqueous extract of *S. macrophylla* seeds (3.72±0.04) mmol/L. The HbA1c percentages in different groups of study subjects also indicate similar trends. Our study shows both the plasma H_2S levels (22.07±0.73) mmol/L and plasma H_2S synthesis activity (0.411±0.005 mmol/100 g) are significantly reduced in the streptozotocin induced diabetic rats.
Conclusions: Although considering a small sample size, it can conclude that the fasting blood glucose levels are inversely related to plasma H_2S levels as well as H_2S synthesis activity in plasma and the extract of *S. macrophylla* is associated with increased plasma H_2S levels with effective lowering of blood glucose in streptozotocin induced diabetic rats.

KEYWORDS
Hydrogen sulphide, Streptozotocin, Diabetes, *Swietenia macrophylla* seed

1. Introduction

In contrast to its role as poison, hydrogen sulfide (H_2S) is now considered as 3rd gas transmitter after nitric oxide and carbon monoxide[1,2]. Though H_2S was first reported to be produced in mammalian tissues in 1982, it is only now emerging as a mediator of important physiologic functions[3]. H_2S is endogenously produced from the amino acid L-cysteine, by two key enzymes that are involved in the trans-sulphuration pathway, cystathionine-b-synthetase and cystathionine γ-lyase. These two enzymes are distributed in a wide range of tissues, including the pancreas, and their expression and activities have been shown to be altered in a variety of pathophysiological conditions[1,4-6]. A third H_2S synthesizing enzyme, 3-mercapto suifer transferase also exits. 3-mercaptosuifer transferase generates H_2S from 3-ercaptosuifertransferese which produced from L-cystine by cysteine aminotransferase[7].

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H_2S regulates several cellular and physiological phenomena such as vasorelaxation, hormone secretion, inflammatory responses and apoptosis[5,7-11]. In the central nervous system, H_2S functions not only as a neuro-modulator, but also as a neuro-protectant against oxidative stress[12].

Recent reports suggest that H_2S play an important role in pathophysiology of many diseases including diabetes mellitus, Alzheimer's disease, hypertension, and cardiac infarction[4,8,12,13]. Recent studies revealed the important role of H_2S in regulation insulin release from the β cells of pancreas. The insulin secretion depends upon K^+ ATP channel opening and closing in the β cells. The main factor controlling the secretion of insulin is the blood glucose concentration. ATP-sensitive K^+-channel determines the resting membrane potential in β cells via a membrane transporter called GLUT-2 and its subsequent metabolism via glycolysis increases intracellular ATP. This blocks K^+ATP causing membrane depolarization and opening of voltage dependant calcium channels, leading to Ca^+ signal which induces insulin secretion. The insulin secretion is depending upon K^+ATP channel opening and closing. H_2S inhibits the closing of K^+ATP and release of insulin. Thereby it prevents exhaustion of β cells and regulates β cell survival. H_2S is K^+ATP channel opener and it has no effect on ATP concentration, may be acting by direct interaction with protein[14,15].

A report suggested pancreatic synthesis of H_2S is markedly elevated in streptozotocin induced rats which have biphasic effect on β-cells. At low concentrations H_2S shown to inhibit insulin release through K^+ATP dependent Ca^{2+} independent mechanism, whereas higher levels induced β-cell death through endoplasmic-reticular-stress-dependent pathways[16].

In addition, the changes in H_2S haemostatics also play a role in the pathogenesis of endothelial injury which developed under the basis of elevated circulation of blood glucose level in diabetes[16].

Previous studies demonstrated the induction of H_2S-producing enzymes in animals treated with the β-cell toxin streptozotocin[17]. The H_2S-producing activity increases in the liver and pancreas of streptozotocin treated diabetic rats whereas the plasma H_2S levels are unchanged[17]. Increases in cystathionine-b-synthetase and cystathionine γ-lyase expression and H_2S production in these diabetic tissues are reversed by insulin treatment, suggesting that this may be a secondary result from hyperglycemia or hypoinsulinemia. However, other studies in this direction have given contradictory findings[17].

The modulation of H_2S production may be a potential therapeutic strategy for these diseases. This possibility led the researchers to investigate H_2S-related substances for treatment of diabetes[9,16]. The cytoprotective effects of H_2S are particularly interesting because exhaustion of β-cells is an important process in the pathogenesis of type 2 diabetes mellitus, and the prevention of β-cell exhaustion may be a new strategy for the treatment of this disease.

Earlier we have reported that *Swietenia macrophylla* (*S. macrophylla*) which is a natural occurring plant are quite effective in controlling the blood glucose levels and the aqueous extract of the seeds have also antioxidant property[18]. We also observed that its helps to regenerate the β-cells in streptozotocin induced diabetic rats[18].

The present investigation was undertaken to evaluate the plasma H_2S levels and H_2S synthesis activity in streptozotocin induced type 2 diabetes rats compared to the healthy controls and also to observe the effect of the aqueous extract of *S. macrophylla* seeds on the above experimental groups.

2. Materials and methods

2.1. Plant material

Seeds of *S. macrophylla* were collected from Botanical Survey of India, Ministry of Environment and Forest, Government Of India, Howrah, 711103 on 4th February, 2010, authenticated by the Botanical Survey of India. A voucher specimen (Ref. no. CNH/I-I/54/2009/Tech.II/154) was deposited at our laboratory for further reference.

2.2. Preparation of extract

The seeds of *S. macrophylla* were separated, washed, shed-dried at room temperature, powdered and sieved through 40 meshes 1 mL of distilled water was added to 200 mg powder to make the solution. After that the solution was centrifuged at 3000 r/min for 15 min. The supernatant was filtrated and collected. The pure extract of *S. macrophylla* was stored in glass vial sealed by air tight lid.

2.3. Animal models

Thirty two wistar rats (180-240 g) were selected for the experiment. The animal were kept under standard condition of 12:12 h light and dark cycle in a polythene cage and fed with standard laboratory diet and water ad libitum. The principle of laboratory animals care and the instructions given by our institutional ethical committee were followed throughout the experiment. Streptozotocin was used for the induction of diabetes by the intraperitoneal route except in normal healthy controls. Hyperglycaemia was induced in overnight fasted adult wistar strain albino rats weighing 180-240 g by a single intraperitoneal injection of 65 mg/kg streptozotocin in a volume of 1 mL/kg body weight[19]. Due to the instability of streptozotocin in aqueous media, the solution was made in citrate buffer (pH 4.5) just before injection[20]. Hyperglycaemia was confirmed by the elevated glucose level in plasma, determined at 48 h after injection. The rats found hyperglycaemic were further screened for the study.

2.4. Experimental design

Animal were divided into four groups of eight rats each and further experiments carried out using six rats in each group.

Group I (Normal Control): Normal rats administered with double distilled water for 30 d.

Group II (Diabetic Control): Diabetic control rats, also administered with distilled water.

Group III (Diabetic rats with metformin): Diabetic rats

administered aqueous extract of metformin (10 mg/kg body weight).

Group IV (Diabetic rats with extract): Diabetic rats administered aqueous extract of *S. macrophylla* seeds daily for 30 d.

2.5. Measurement of H_2S concentration in plasma

H_2S concentration in plasma was measured following the method described earlier[11]. Plasma was collected from rat blood before sacrifice followed by centrifugation. 75 μL plasma was mixed with 250 mL 1% (w/v) zinc acetate and 425 mL distilled water in a tube. Then 20 mmol/L N-dimethyl-p-phenylenediamine sulphate in 7.2 mmol/L HCl (133 mL) and 30 mmol/L $FeCl_3$ in 1.2 mmol/L HCl (133 mL) were also added to the test tube for 10 min incubation at room temperature. The protein in the plasma was removed by adding 250 mL of 10% tricholoacetic acid to the reaction mixture and pelleted by centrifugation at 3000 r/min for 15 min. The absorbance of the resulting solution at 670 nm was measured with a spectrophotometer. All samples were assayed in duplicate and concentration in the solution was calculated against a calibration curve of NaHS (3.125-250 mmol/L).

2.6. Measurement of H_2S synthesizing activity

H_2S synthesizing activity in plasma is measured by following method of Kun Qu *et al.* with some modification. One hundred μL of plasma is taken in a vial with 0.3 mL of 1% zinc acetate. Throughly mixed capped with airtight cap incubated at 37 °C for 90 min. Half mL of 50% trichloroacetic acid injected into the reaction mixture through the cap and incubated for another 60 min. Then 50 μL of mmol/L N, N-dimethyl-p-henylenediamine sulphate in 7.2 mol/L HCl and 50 μL of 30 mmol/L $FeCl_3$ in 1.2 mol/L HCl were added into the tube through the cap. Incubated for further 20 min at 37 °C and absorbance was measured at 670 nm using spectrophotometer. H_2S concentration was taken as 30% of the NaHS concentration in the calculation. The calibration curve was linear from 0 to 320 mmol/L NaHS or 96 mmol/L H_2S.

2.7. Measurement of other biochemical parameters

Measurement of blood glucose, urea, creatinine, SGOT, SGPT and HbA1c were done using standardised reagent kits (Ranbaxy).

2.8. Statistical analysis

Statistical analysis have done using Microsoft excel, Office 2003. Values were expressed by mean±SD. $P<0.05$ were considered statistically significant.

3. Results

The results of different biochemical parameters in the current study have been depicted in Table 1. Fasting blood glucose level (7.74±0.02) mmol/L was significantly increased in diabetic rats (Group II) in comparison to healthy control ones (Group I) (4.04±0.02) mmol/L. The glucose levels are significantly lowered in the rats treated with metformin (5.48±0.03) mmol/L as well as with aqueous extract of *S. macrophylla* seeds (3.72±0.04) mmol/L. In the current study the plasma H_2S levels in the control group was within the range of 23.45 to 26.83 mmol/L (25.00±0.14) mmol/L.

The current study observed both the plasma H_2S levels (22.07±0.73) mmol/L and plasma H_2S synthesis activity (0.411±0.005) mmol/100 g were significantly reduced in the streptozotocin induced diabetic rats. Whereas the plasma H_2S levels in the rats treated with metformin [Group III, (25.97±0.1) mmol/L] and the aqueous extract of *S. macrophylla* (Group IV, 36.85±0.64 mmol/L) was significantly increased compared with diabetic rats. The plasma H_2S synthesis activity of healthy control, diabetic control, diabetes on metformin and extract were (51.6±0.08), (41.1±0.05), (47.5±3.0) and (70.2±0.09) mmol/100 g protein respectively. Therefore, our study results show both plasma H_2S level and H_2S synthesis activity is significantly enhanced with the reduction blood glucose levels (Figures 1-4).

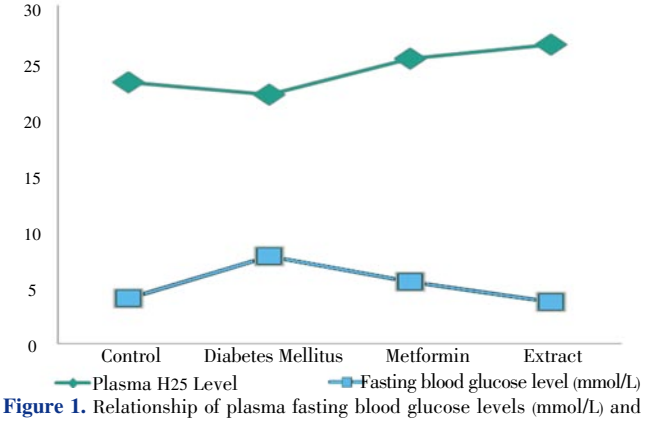

Figure 1. Relationship of plasma fasting blood glucose levels (mmol/L) and plasma H_2S concentration (mmol/L).

Table 1

Biochemical parameters of the study subjects.

Parameters	Normal Control	Diabetic Control	Diabetic rats with Metformin	Diabetic rats with extract
Plasma H_2S Levels (mmol/L)	25.14±1.69	22.07±0.73	25.97±0.10	36.85±0.64[a]
Plasma H_2S Synthesis Activity (mmol/100 g of protein)	51.60±0.08	41.10±0.05	47.50±3.00	70.20±0.09[b]
Body weight (g)	105.78±4.31	107.30±4.91	108.03±5.49	108.32±4.20
Fasting blood glucose (mmol/L)	4.04±0.02	7.74±0.02	5.48±0.03	3.72±0.01
HbA1c (%)	5.50±0.06	5.80±0.10	5.00±0.06	3.80±0.12
Urea (mg/dL)	22.95±2.43	24.45±3.40	20.05±2.11	19.56±1.40
Creatinine (mg/dL)	0.50±0.09	0.70±0.07	0.67±0.01	0.40±0.02
SGOT (U/L)	42.22±3.59	46.83±4.79	46.66±9.24	42.66±1.96
SGPT (U/L)	38.91±3.20	42.16±3.86	39.83±3.76	34.16±2.85[a]

Paired two tail student's T test was done. Values are expressed as mean±SD. Indicate the level of significance in comparison to the healthy controls (Group I). [a]$P<0.05$; [b]$P<0.01$.

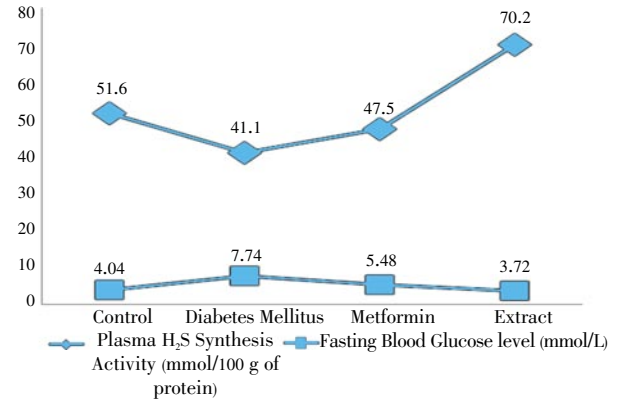

Figure 2. Relationship of plasma fasting blood glucose levels (mmol/L) and plasma H_2S synthesis activity (mmol/100 g of protein).

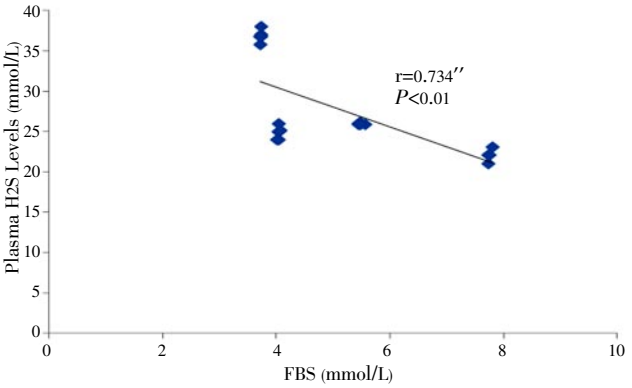

Figure 3. Correlation of fasting blood glucose and H_2S levels in streptozotocin induced diabetic rat.

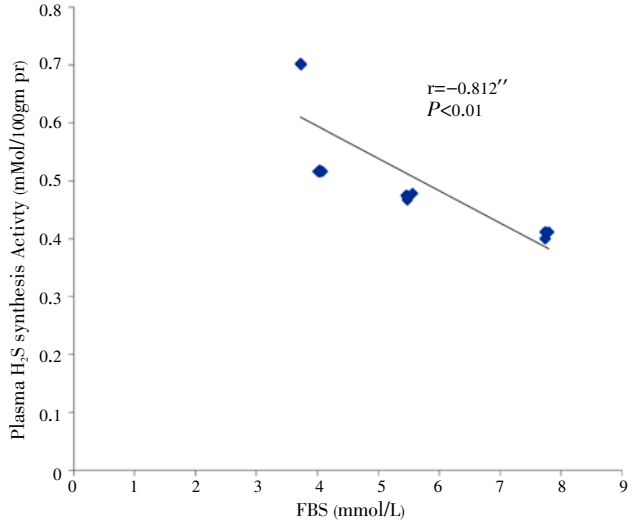

Figure 4. Correlation of fasting blood glucose and H_2S level synthesis activity in streptozotocin induced diabetic rat.

4. Discussion

The current study showed fasting blood glucose level significantly increased in diabetic rats compared to healthy controls along with the glucose levels being significantly lowered in the rats treated with metformin as well as with aqueous extract of *S. macrophylla* seeds and this conforms with the findings of our previously reported study[18]. Even the glycated haemoglobin percentages in different groups of study subjects also had similar trends of our previously reported study[18].

In the current study the plasma H_2S levels in the control group is within the range and it is in good agreement with study conducted elsewhere[7]. However, all studies didn't conform to the findings of the current study and had reported some contradictory findings. One of the study reported that the streptozotocin induced diabetes in the rat is associated with enhanced tissue hydrogen sulphide biosynthesis[17]. Another study suggested that H_2S synthesis is progressively reduced as diabetic pathology increased[21].

The current study shows that both plasma H_2S levels and plasma H_2S synthesis activity are significantly reduced in the streptozotocin induced diabetic rats. Whereas the plasma H_2S levels in the rats treated with metformin and the aqueous extract of *S. macrophylla* is significantly increased compared to diabetic rats. The extract treated rats having lowest blood glucose levels, show highest levels of H_2S and H_2S synthesis activity in plasma. Plasma H_2S synthesis activity also shows similar observations. Therefore, our study results show both plasma H_2S level and H_2S synthesis activity is significantly enhanced with the reduction blood glucose levels. Earlier study described that the blood glucose level is significantly higher and insulin level significantly lower in animal treated with NaHS and H_2S donar compared to the control group. According to JIA, H_2S concentration reported to be higher in Juker diabetic rats compare to the juker lean rats. According to their study H_2S increases glucose level and decreases insulin levels[22]. In our study both H_2S levels and H_2S synthesis activity is increased with decrease of plasma glucose levels and these are well correlated. H_2S levels especially under the effect of the extract is associated with further decrease in the plasma glucose concentration Previously we have reported that the aqueous extract have significantly antioxidant effect and also helps to regenerate the β-cells. Increased plasma H_2S level may also contribute for regeneration of the β-cells observed in our earlier study. The higher H_2S levels observed in our study may be due to other factors involved in the H_2S bioavailability in the vasculature.

Although considering a small sample size, we conclude that the fasting blood glucose levels are inversely related to plasma H_2S levels as well as H_2S synthesis activity in plasma and the extract of *S. macrophylla* is associated with increased plasma H_2S levels with effective lowering of blood glucose in streptozotocin induced diabetic rats.

Conflict of interest statement

We declare that we have no conflict of interest.

Comments

Background

The application of H_2S in diagnosing the extent of progression of diabetes is the insight of the current study

highlights the link of H_2S and diabetes. Also the role of herbal plant product swietenia macrophylla is being studied as a protector of damage in pancreatic tissue leading to progression of diabetes.

Research frontiers

The relationship of H_2S with pancreatic tissue efficiency in production and synthesis of insulin is being the current trend and the importance and cutting edge finding of this research study. Also the relationship of *S. macrophylla* with H_2S is established which is not reported earlier.

Related reports

Earlier studies have been done but no concrete findings have been reported and there was not extreme analysis on diabetes with H_2S as reported in the current study. The materials and methods are good and it is done in a research based university. The finding/s of this study would be of immense importance for re-thinking the role of H_2S in diabetes.

Innovations and breakthroughs

The current study is innovative and gives us an idea to explore further and to re-think why and how H_2S affects pancreatic tissue and its role in insulin release and its importance in diabetes and hypoglycemic states.

Applications

Point of care management of diabetes with application of H_2S being used as a marker of its disorder and linking with herbal product is of significance as it does not have any side-effects and contraindications compared to the traditional ones.

Peer review

Over all the paper is very informative and gives very scientific information, which makes us to rethink about the relationship of H_2S and diabetes mellitus.

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