

Original Article

PROTECTING OUTCOME OF ETHYL ACETATE FRACTION OF AQUEOUS-METHANOL: 40:60 EXTRACT OF SEED OF *MYRISTICA FRAGRANS* HOUTT. IN STREPTOZOTOCIN-INDUCED DIABETIC RAT: A DOSE DEPENDENT STUDY

ANKITA DEY, ADRIJA TRIPATHY, DEBIDAS GHOSH*

¹Molecular Medicine and Nutrigenomics Research Laboratory, Department of Bio-Medical Laboratory Science and Management, Vidyasagar University, Midnapore 721102, West Bengal, India
Email: debidas_ghosh@yahoo.co.in

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ABSTRACT

Objective: The investigation has been conducted to find out the threshold dose of ethyl acetate fraction prepared from aqueous-methanol (40:60) extract of seed of *Myristica fragrans* (Houtt.) from the dose dependent experiment by noting the remedial effects of the said fraction on different complications developed in streptozotocin induced diabetic rat.

Methods: Diabetic condition was made by single intramuscular injection of streptozotocin at a dose of 4 mg/0.1 ml citrate buffer/100 g body weight. Treatment was conducted at different doses for 28 d on diabetic rat. Efficacy of the fraction on fasting blood glucose (FBG) and serum insulin levels, activities of key carbohydrate metabolic enzymes such as hexokinase, glucose-6-phosphate dehydrogenase, glucose-6-phosphatase of hepatic and skeletal muscle tissue as well as antioxidative enzymes like catalase, superoxide dismutase (SOD) in connection with the levels of oxidative stress end products such as thiobarbituric acid reactive substances (TBARS), conjugated diene (CD) in hepatic and renal tissue and gene expression of hexokinase-1, SOD, Bax, Bcl-2 in hepatic tissue were assessed.

Results: After different doses of fraction treatment to the diabetic animals; FBG, serum insulin levels and activities of carbohydrate metabolic enzymes i.e. hexokinase, glucose-6-phosphate dehydrogenase and glucose-6-phosphatase were rectified towards the control at the level of $p < 0.05$. There is a significant ($p < 0.05$) recovery in the activities of antioxidant enzymes such as catalase and SOD in respect to the vehicle treated diabetic group. Oxidative stress end products such as CD and TBARS levels were increased significantly ($p < 0.05$) in vehicle treated diabetic rats and was rectified significantly ($p < 0.05$) after fraction treatment at the dose of 10 mg or 20 mg or 40 mg fraction treated group. Gene expression of hexokinase-1, superoxide dismutase, bax and bcl-2 was rectified towards vehicle treated control significantly at the level of $p < 0.05$ after treatment of different doses of fractions to the diabetic animals.

Conclusion: The study indicates that the seeds of *Myristica fragrans* at the dose of 20 mg/100 g body weight possess most effective outcome to manage diabetic disorders in streptozotocin induced diabetic rats.

Keywords: Antidiabetic, Streptozotocin, Antioxidant, *Myristica fragrans*, Fasting blood glucose, Serum insulin, and Gene expression

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INTRODUCTION

Diabetes mellitus has been termed as a set of metabolic dysfunctions associated with chronic hyperglycemia due to impaired secretion and/or action of insulin which results in distortion in the metabolism of carbohydrate, fat and protein [1, 2]. Diabetes mellitus has been established to be high level of oxidative stress injury and accordingly an variation in the body's antioxidant defense system [3, 4]. It is a common metabolic disorder with micro- and macrovascular complication that results morbidity and mortality. It is considered as one of the five leading reasons of death in the world [5, 6]. A number of man-made drugs such as glibenclamide, biguanide and sulfonylurea are now available to diminish the threat of diabetes mellitus but these drugs cause side effects [7]. As a consequence, there is a need to explore a new class of remedial compounds to defeat those problems [8]. The World Health Organization (WHO) recommended the consideration of plant and folk medicine for management of some diseases [9]. From the literature review, over 800 plant species have been noted with antidiabetic efficacy [10]. Various native remedial plants and extracts from different parts of these plants like root, leaf, flower, seed etc. have been [11-13] investigated for the management of diabetes.

Nutmeg (*Myristica fragrans* Houtt.), a tropical evergreen tree is the source of the high rate healing spices, nutmeg (endosperm) and mace (the reddish aril) with massive phytochemical variety [14]. Current information on its anti-diabetic, anti-leukaemic, anti-bacterial, anti-viral effects and other biological activities [14-16] specify its massive beneficial potential.

There is no complete work regarding the anti-diabetic and antioxidative activities of the seed of *Myristica fragrans* (*M. fragrans*) from the standpoint of biochemical and molecular studies. Hence, the present study has been conducted to explore the effective dose of ethyl-acetate fraction of aqueous-methanolic (40:60) extract of seed of *M. fragrans* in streptozotocin induced diabetic male rat with special reference to correction of carbohydrate metabolic disorders from genomic point of view.

MATERIALS AND METHODS

Preparation of ethyl acetate fractionation from aqueous-methanol (40:60) extract of seed of *M. fragrans*

The seeds of *M. fragrans* were collected (August-September, 2015) from the local market place of Paschim Medinipore town, Midnapore, West Bengal, India and identified by Prof. Ram Kumar Bhakat, taxonomist in the Department of Botany and Forestry, Vidyasagar University, Midnapore. The voucher specimen was preserved there, having Ref. No.-(BMLSM-11/2016). Extraction and fractionation of seed was done by standard as per our earlier report [17]. This fraction residue was suspended in tween 80 and water at a ratio of 1:19, which was used for treatment.

Selection of animal and animal care

This investigational work was conducted by using thirty matured Wistar strain normoglycemic male albino rats, age near about 3 mo, weighing about 100 ± 10 g, six rats were housed in each hygienic and

dry polypropylene made cage. Earlier to conducting the tests, all rats were acclimated for 15 d in laboratory situation. Standard atmosphere in the animal house was maintained with dark-light cycle (12h: 12h) and room temperature at 28 ± 2 °C with 45%-60% humidity. Each rat had open access to standard food as well as water *ad libitum* for specific period. The principle of laboratory animal care and instruction given by our Institutional Ethic Committee (IEC) [IEC/9/2/18, dated 02/02/2018] were followed during the experiment.

Chemicals

Sigma, USA made streptozotocin (STZ) was purchased for the conduction of research work. Other chemicals like adenosine tri phosphate (ATP), nicotinamide adenine dinucleotide phosphate (NADP), 2-[4-(2-hydroxyethyl) 1-piperazinyl] ethane sulphonic acid (HEPES) were used from Sigma-Aldrich Diagnostic Ltd. or Sisco Research Laboratory (SRL), India. Kits for the assessment of serum insulin level was purchased from RayBiotech, Norcross, USA. Isolation kit from RNA and 'Transcriptor first strand cDNA synthesis kit' were purchased from Roche Diagnostic, Mannheim, Germany for real time PCR study.

Induction of diabetes mellitus in rat

In connection to our prior work, the projected dose of streptozotocin (STZ) which generates diabetic state in adult rat was selected [18]. In concise, overnight fasted rats were subjected to a single intramuscular injection of STZ at the dose of 4 mg/0.1 ml of citrate buffer/100 g body weight/rat that generate diabetes. The fasting blood glucose (FBG) level was in between 300-340 mg/dl after 48h of STZ injection. After the 7th day of STZ injection, the FBG level was measured once more to observe the constancy of the diabetic state. In this approach, twenty four rats were made as diabetic.

Animal treatment

Five homogeneous groups were prepared by using thirty rats. Ethyl acetate fraction of aqueous-methanol (40:60) extract of seed of *M. fragrans* was given from the 7th day of post injection phase of STZ and was considered as 1st day of treatment. The duration of treatment was 28 d.

The rats were alienated as follows into five groups:

Group-I: Vehicle treated control: (Received 0.5 ml of 3.0% Tween 80/100 g body weight/day).

Group-II: Vehicle treated diabetic: (Received 0.5 ml of 3.0% Tween 80/100 g body weight/day).

Group-III: Ethyl acetate fraction 10 mg: (Received 10 mg/0.5 ml of 3.0% Tween 80/100 g body weight/day).

Group-IV: Ethyl acetate fraction 20 mg: (Received 20 mg/0.5 ml of 3.0% Tween 80/100 g body weight/day).

Group-V: Ethyl acetate fraction 40 mg: (Received 40 mg/0.5 ml of 3.0% Tween 80/100 g body weight/day).

Every day, the oral dose of the above-mentioned fraction was given to the overnight fasted rats in the morning (at 11:00 am). After 2 h of fraction treatment; food was given to the rats. The feed box was cleaned in the evening (at 7:00 pm). By using single touch glucometer, fasting blood glucose (FBG) level was measured in all the groups on every 7th d [19].

Treatment of fraction or vehicle to the animals was performed by forced feeding with gavage. On 29th day of experiment considering treatment of fraction as 1st day of the experiment schedule, all the animals were sacrificed at fasting state by euthanasia followed by decapitation after recording the final body weight. Blood was collected from dorsal aorta by a syringe and the serum was separated by centrifugation for the measurement of insulin level. The liver, kidney, skeletal muscle (Gastrocnemius) were dissected out and stored at -20 °C for the measurement of different relevant parameters in this concern. Pancreas was collected and was placed in Bouin's fluid for histological analysis.

Measurement of FBG level

At the grouping time of all the animals, FBG level was measured. Further FBG levels were recorded throughout the experiment at 7 d

of interval. Blood was collected from the tail vein by syringe following warm up the tail. Single touch glucometer was used for the measurement of FBG level [19].

Assay of serum insulin

Serum insulin level was measured using solid phase conjugated sandwich ELISA kit for rat. The level of insulin was expressed in terms of $\mu\text{U/ml}$ [20].

Assay of hexokinase activity

In a spectrophotometer cuvette 0.9 ml of assay mixture (3.7 mmol glucose, 7.5 mmol MgCl_2 , 11 mmol thioglycerol and 45 mmol HEPES, pH 7.5), 0.01 ml glucose-6-phosphate dehydrogenase, 0.01 ml NADP and 0.03 ml ATP were added and mixed well. To it 0.6 ml of tissue supernatant was added to the cuvette and mixed immediately, then read at 340 nm. The subsequent six readings were recorded at 30 sec interval. The enzyme activity was determined on the basis of reduction of NADPH which was measured spectrophotometrically at 340 nm. One unit of hexokinase activity was expressed in unit/g of tissue [21].

Biochemical assay of glucose-6-phosphate dehydrogenase activity

The glucose-6-phosphate dehydrogenase activity was measured spectrophotometrically following the standard method [22]. Tissue was homogenized in ice-cold 0.1 M phosphate buffer saline (pH-7.4) at the tissue concentration of 50 mg/ml. In spectrophotometric cuvette, 0.3 ml of 1M Tris chloride buffer (pH-7.5), 0.3 ml of 2.5×10^{-2} M glucose-6-phosphate, 0.1 ml of 2×10^{-3} M NADP and 0.3 ml of 0.2 M MgCl_2 and 0.3 ml of ice cold tissue homogenate were taken. The rate of change of absorbance at 340 nm was recorded. One unit of enzyme activity define as that quantity which catalyses the reduction in 1 μM of NADP per minute.

Biochemical assay of glucose-6-phosphatase activity

The hepatic glucose-6-phosphatase activity was measured according to standard protocol [23]. Tissue was homogenized in ice cold of 0.1 M phosphate buffer saline (pH 7.4) at the tissue concentration of 50 mg/ml. In a calibrated centrifuge tube, 0.1 ml of 0.1 M glucose-6-phosphate solution and 0.3 ml of 0.5M maleic acid buffer (pH 6.5) were taken and brought to 37 °C in water bath for 15 min. The reaction was stopped with 1 ml of 10% trichloroacetic acid (TCA) followed by chilling in ice and centrifuge at 3000g for 10 min. The optical density was noted at 340 nm. The enzyme activity was expressed as μg of inorganic phosphate liberated per g of tissue.

Biochemical assay of catalase activity

For the evaluation of catalase activity, liver and kidney tissues were homogenized in 0.05 M Tris-HCl buffer solution (pH-7.0) at the tissue concentration of 50 mg/ml. These homogenized samples were centrifuged at $10000 \times g$ at 4 °C for 10 min. In spectrophotometer cuvette, 0.5 ml of 0.00035 M H_2O_2 and 2.5 ml of distilled water were added and mixed. Readings of absorbance was noted at 240 nm before the addition of supernatant. Then, supernatant was added at a volume of 40 μl to the cuvette and the kinetics was measured by recording successive six readings at 30 sec interval. Finally the result was expressed as μM of H_2O_2 consumed/mg of tissue/min [24].

Assessment of superoxide dismutase (SOD) activity

Activity of SOD in the tissue sample was estimated by measuring the percentage inhibition in the pyrogallol auto oxidation by superoxide desmutase according to standard method [25]. In a spectrophotometric cuvette, 2.04 ml of 50 mmol Tris buffer (pH-8.2), 20 μl of sample and 20 μl of pyrogallol were taken and the absorbance was observed in spectrophotometer at 420 nm for 3 min. One unit of superoxide was defined as the enzyme activity that inhibits the auto oxidation of pyrogallol by 50%.

Estimation of lipid peroxidation from the concentration of thiobarbituric acid reactive substances (TBARS) and conjugated diene (CD)

Tissues were homogenized at the concentration of 50 mg/ml in 0.1 M of ice-cold phosphate buffer (pH-7.4) and the homogenates were centrifuged at $10000 \times g$ at 4 °C for 5 min. Each supernatant was used for the estimation of TBARS and CD. For the measurement of TBARS, the homogenate mixture of 0.5 ml was mixed with 0.5 ml of normal saline (0.9 g % NaCl) and 2 ml of TBA-TCA mixture (0.392 g thiobarbituric acid

in 75 ml of 0.25(N) HCl with 15 gm trichloroacetic acid. The volume of the mixture was made up to 100 ml by 95 % ethanol and boiled at 100 °C for 10 min. This mixture was then cooled at room temperature and centrifuged at 4000 × g for 10 min. The whole supernatant was transferred in spectrophotometer cuvette and read at 535 nm [26].

Quantification of the CD was performed by standard method [27]. The lipids were extracted with chloroform-methanol (2:1) mixture followed by centrifugation at 1000 × g for 5 min. The chloroform layer was evaporated to dryness under a stream of nitrogen. The lipid residue was dissolved in 1.5 ml of cyclohexane and the absorbance was noted at 233 nm to measure the amount of hydro peroxide formed.

RNA isolation and complementary DNA synthesis

Whole existing RNA in hepatic tissue was extracted using kit (Roche Diagnostic, Mannheim, Germany), and from that extracted RNA, cDNA was synthesised using 'Transcriptor First Strand cDNA Synthesis Kit' (Roche Diagnostic) [28].

Quantitative reverse transcription polymerase chain reaction (qRT-PCR) analysis

For the evaluation of gene expression of Hexokinase-I, SOD, Bax, Bcl-2 and β-actin genes of hepatic tissues by using Light Cycler 480 II (Roche Diagnostic) was used [28].

Statistical analysis

Analysis of Variance (ANOVA) followed by 'Multiple comparison two-tail't' test' was used for statistical analysis of collected data [29]. All the values were indicated by mean±SEM, (n = 6). For statistical analysis of data, differences were considered significant at p<0.05.

RESULTS

Fasting blood glucose level

Fasting blood glucose level was significantly elevated (p<0.05) in STZ-induced diabetic rats in respect to vehicle treated control group. Treatment of ethyl acetate fraction of aqueous-methanol: : 40:60 extract of seed of *M. fragrans* at the dose of 10 mg or 20 mg or 40 mg/100 g of body weight for 28 d to the diabetic rats resulted a significant diminution (p<0.05) in FBG level when comparison was made with vehicle treated diabetic group. From the comparative analysis of the data it was observed that 20 mg or 40 mg fraction treated group resulted a significant diminution (p<0.05) of fasting blood glucose level in respect to 10 mg fraction treated diabetic animals though no significant variation was noted in the level of the said parameter between 20 mg and 40 mg fraction treated diabetic groups indicated that 20 mg dose is the threshold dose in this concern (table 1).

Table 1: Dose dependent effect of ethyl acetate fraction of seed of *M. fragrans* on FBG level in STZ-induced diabetic male albino rat

Group	Fasting blood glucose level (mg/dl)				
	1st d (7th d of STZ injection)	8th d	15th d	22nd d	28th d
Vehicle treated control	70.21±2.8 ^a	71.07±3.1 ^a	69.19±3.3 ^a	72.21±3.1 ^a	71.71±2.6 ^a
Vehicle treated diabetic	329.02±4.6 ^b	334.51±5.1 ^b	337.12±5.6 ^b	337.17±4.8 ^b	339.24±4.2 ^b
Ethyl acetate fraction 10 mg	333.3±4.6 ^b	337.91±5.1 ^b	332.42±3.8 ^b	319.14±3.5 ^b	216.24±4.4 ^c
Ethyl acetate fraction 20 mg	337.54±4.3 ^b	236.42±4.2 ^d	176.24±3.4 ^d	136.12±3.8 ^d	114.09±2.1 ^d
Ethyl acetate fraction 40 mg	334.71±4.2 ^b	252.02±4.4 ^d	208.91±4.6 ^d	152.21±4.5 ^d	146.91±3.6 ^d

Value represents mean±SEM (n=6). Values with different superscripts (a, b, c, d) in each vertical column differ from each other significantly, p<0.05.

Serum insulin

Serum insulin level was decreased significantly (p<0.05) in STZ-induced diabetic group in respect to vehicle treated control group. Ethyl acetate fraction of aqueous-methanol: : 40:60 extract of seed of *M. fragrans* treatment to diabetic animals at the dose of 10 mg or 20 mg or 40 mg/100 g of body weight for 28 d resulted a significant elevation (p<0.05) in serum insulin level when compare with vehicle

treated diabetic group. After comparison of the data among different fraction treated groups it was observed that 20 mg or 40 mg/100 g of body weight showed more promising results than other fractions treated groups though the level of serum insulin was not resettled to the control level. Insignificant variation in serum insulin level was noted between 20 mg and 40 mg/100 g of body weight fraction treated diabetic groups which suggest the threshold dose in this concern is 20 mg/100 g body weight (fig. 1).

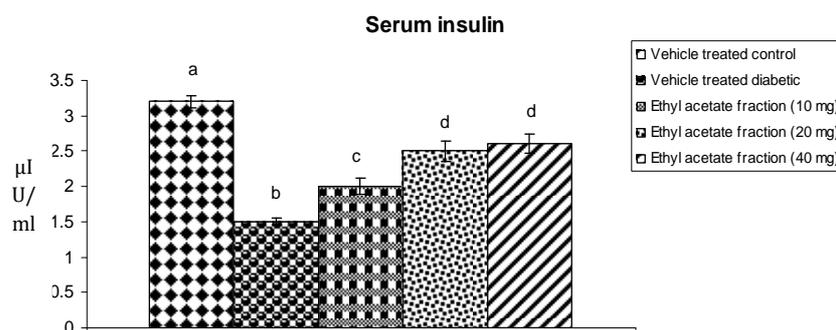


Fig. 1: Significant recovery in the level of serum insulin after administration of ethyl acetate fraction of seed of *M. fragrans* at different dose treated groups in STZ-induced diabetic rat. Bars were expressed as mean±SEM (n=6). Bars with different superscripts (a, b, c, d) differ from each other significantly, p<0.05

Activities of hexokinase and glucose-6-phosphate dehydrogenase in liver and skeletal muscle

Activities of hexokinase and glucose-6-phosphate dehydrogenase in liver and skeletal muscle tissues were diminished significantly (p<0.05) in STZ-induced diabetic group in compare with the vehicle

treated control group. After the treatment of ethyl acetate fraction of seed of *M. fragrans* at the dose of 10 mg or 20 mg or 40 mg/100 g of body weight for 28 d to the STZ-induced diabetic animals resulted a significant elevation (p<0.05) in the activities of these enzymes in respect to vehicle treated diabetic group. Results focused that 20 mg or 40 mg dose significantly effective (p<0.05) in compare with 10

mg dose of ethyl acetate fraction though the levels of the said sensors were not resettled to the control level. There was no significant variation in the levels of these parameters between 20 mg and 40 mg/100 g of body weight fraction treated diabetic groups (fig. 2).

Assessment of glucose-6-phosphatase activity

Activities of glucose-6-phosphatase in hepatic and skeletal muscular tissues were increased significantly ($p < 0.05$) in the diabetic animals in compare with the vehicle treated control animals. Vehicle treated

diabetic animals treated with different dose of ethyl acetate fraction of seed of *M. fragrans* resulted a significant diminution ($p < 0.05$) in the activities of glucose-6-phosphatase in respect to the vehicle treated diabetic group. Comparative analysis of the data in the activities of the enzyme indicated that 20 mg and 40 mg/100 g of body weight dose treated group showed significant effective result ($p < 0.05$) in compare with 10 mg/100 g of body weight dose treated group. There was no significant variation in the levels of these parameters between 20 mg and 40 mg/100 g of body weight fraction treated diabetic groups (fig. 2).

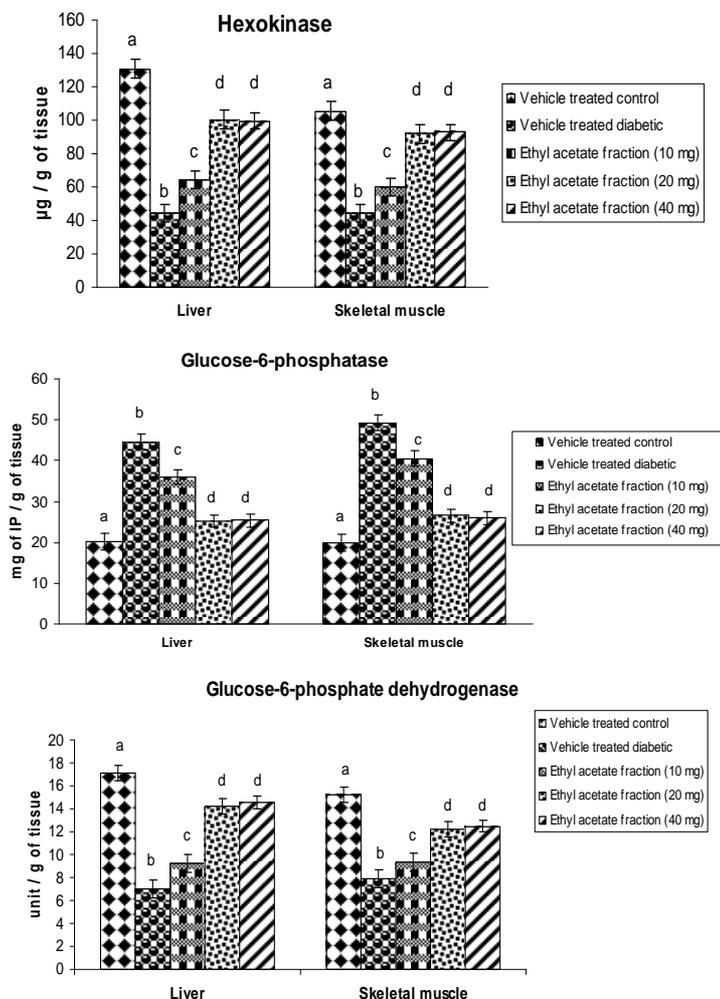


Fig. 2: Resettlement in the activities of hexokinase, glucose-6-phosphate dehydrogenase and glucose-6-phosphatase in liver and skeletal muscle after treatment of ethyl acetate fraction of aqueous-methanol extract of seed of *M. fragrans* at different doses in vehicle treated diabetic rat. Values were expressed as mean \pm SEM, n=6. Bars with different superscripts (a, b, c, d) differ from each other significantly, $p < 0.05$

Catalase and SOD activities in liver and kidney tissues

Activities of catalase and SOD in liver and kidney tissues were significantly diminished ($p < 0.05$) in STZ treated diabetic group in compare with the vehicle treated control group. Administration of ethyl acetate fraction of seed of *M. fragrans* at the dose 10 mg/100g of body weight for 28 d to the diabetic rats resulted a significant elevation ($p < 0.05$) in the activities of catalase and SOD in liver and kidney tissues in compare with vehicle treated diabetic group. In contrast, after 28 d treatment of said fraction at the dose of 20 mg or 40 mg/100g body weight to diabetic rats, a significant recovery ($p < 0.05$) was noted in the activities of the concerned parameters in respect to other doses of ethyl acetate fraction. The levels of these parameters were insignificantly differed when the results were compared between 20 mg and 40 mg/100 g of body weight fraction treated groups though the levels were not resettled to the control level (fig. 3).

Quantification of TBARS and CD in liver and kidney tissues

Quantity of TBARS and CD were significantly increased ($p < 0.05$) in liver and kidney tissues of STZ induced diabetic group in respect to the vehicle treated control group. Treatment of ethyl acetate fraction of seed of *M. fragrans* at the dose of 10 mg or 20 mg or 40 mg/100 g body weight/day for 28 d to the diabetic rats resulted a significant diminution ($p < 0.05$) in the levels of these parameters in respect to the vehicle treated diabetic group. A significant recovery ($p < 0.05$) was observed in 20 mg or 40 mg fraction treated group in compare with 10 mg fraction treated group though the levels were significantly less ($p < 0.05$) than the control level. There was no significant difference between 20 mg and 40 mg/100 g of body weight fraction administered groups in the levels of TBARS and CD in liver and kidney tissues (fig. 4).

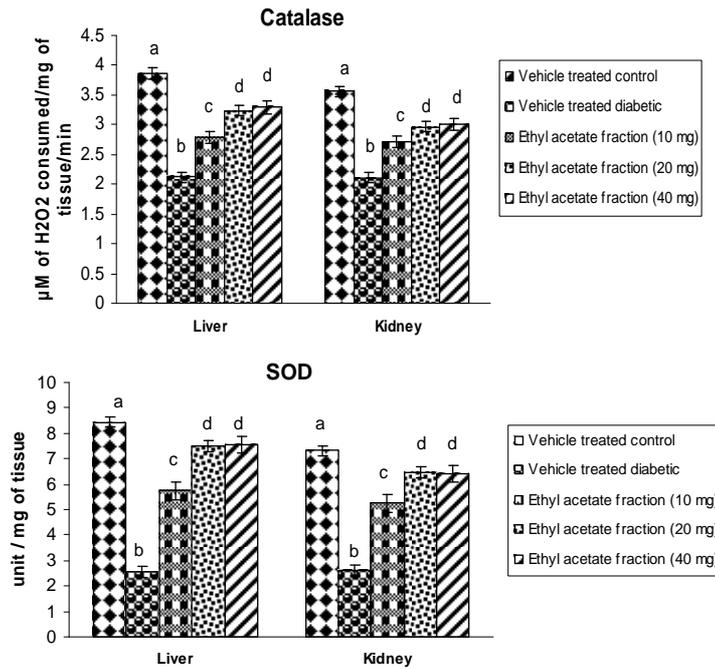


Fig. 3: Amelioration in the activities of catalase and SOD in hepatic and renal tissue after treatment with ethyl acetate fraction of hydro-methanol extract of seed of *M. fragrans* at different doses in STZ-induced diabetic rat. Values were expressed as Mean \pm SEM, n=6. Bars with different superscripts (a, b, c, d) differ from each other significantly, $p < 0.05$

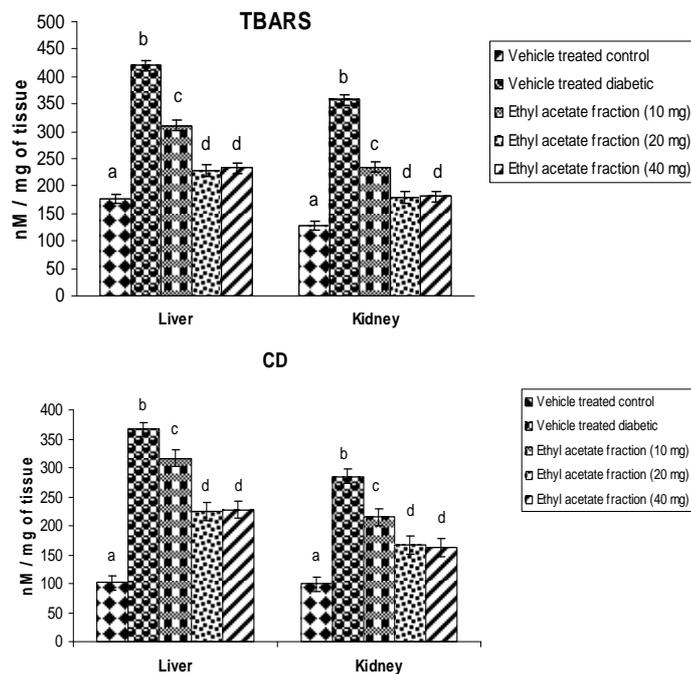


Fig. 4: Remedial effect of ethyl acetate fraction of seed of *M. fragrans* at different doses on TBARS and CD levels in hepatic and renal tissues in comparison to the vehicle treated diabetic rat. Values were represented mean \pm SEM (n=6). Bars with different superscripts (a, b, c, d) differ from each other significantly, $p < 0.05$

Gene expression pattern of hexokinase-1, SOD, Bax, Bcl-2 in hepatic tissue

Hexokinase-1, SOD and Bcl-2 gene expression levels were decreased significantly ($p < 0.05$) in vehicle treated diabetic rats and this genetic levels were further resettled after ethyl acetate fraction of seed of *M. fragrans* treatment to the diabetic rats at the dose of 10 mg or 20 mg or 40 mg/100 g body weight/day for 28 d.

Gene level of Bax was significantly increased ($p < 0.05$) in vehicle treated diabetic group and it was further recovered in different doses fraction treated groups. A significant recovery ($p < 0.05$) was noted in 20 mg or 40 mg fraction treated group in contrast with 10 mg fraction treated group though the levels were significantly less ($p < 0.05$) than the control level. There was no significant difference between 20 mg and 40 mg/100 g of body weight fraction administered groups (fig. 5.1-5.3).

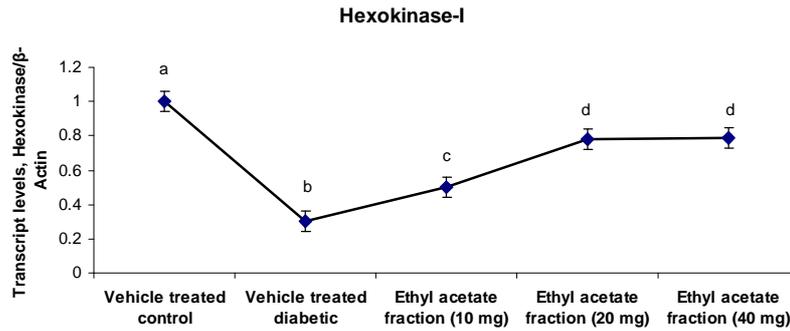


Fig. 5.1: Line diagram of qRT-PCR analysis of gene expression of hexokinase-I in hepatic tissue after administration of ethyl acetate fraction of aqueous-methanol (40:60) extract of seed of *M. fragrans* in diabetic animals. Values were expressed as mean \pm SEM (n=6). Values with different superscripts (a, b, c, d) differ from each other significantly, p<0.05

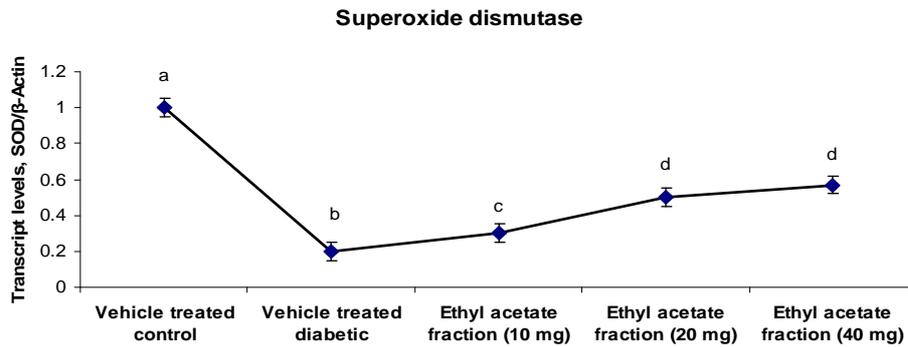


Fig. 5.2: Gene expression pattern of SOD in hepatic tissue after oral administration of ethyl acetate fraction of aqueous-methanol (40:60) extract of seed of *M. fragrans* to vehicle treated diabetic rat. Values were expressed as mean \pm SEM, n=6. Values with different superscripts (a, b, c, d) differ from each other significantly, p<0.05

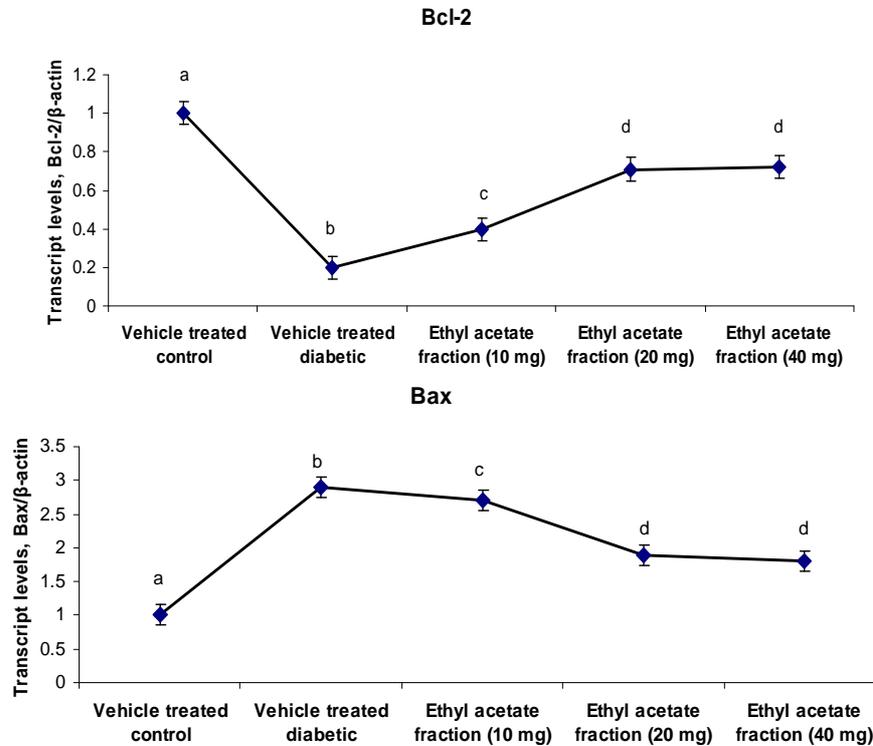


Fig. 5.3: Investigation of Bax, Bcl-2 gene expression through qRT-PCR in hepatic tissue after treatment with ethyl acetate fraction of aqueous-methanol (40:60) extract of seed of *M. fragrans* to vehicle treated diabetic rat. Values were expressed as mean \pm SEM, n=6. Values with different superscripts (a, b, c, d) differ from each other significantly, p<0.05

DISCUSSION

The dose dependent study has been performed to find out the threshold dose of ethyl acetate fraction of seed of *M. fragrans* for the management of diabetic disorders in STZ-induced diabetic rats. Three different doses i.e. 10 mg, 20 mg dose and 40 mg were adopted in the present study where 20 mg showed effective diminution of FBG level in respect of vehicle treated diabetic rats. So, it may be stated that the extract stimulates the β -cells of pancreas for the secretion of insulin or it has the capacity for restoration of pancreatic β -cells as observed by others, using separate plant products [30]. The above findings have been further supported from the outcome of serum insulin level which was increased by the treatment of ethyl acetate fraction of seed of *M. fragrans* at the dose of 20 mg and 40 mg/100 g of body weight in respect to diabetic animal. The dose at the level of 20 mg and 40 mg/100 g of body weight of ethyl-acetate fraction of *M. fragrans* seed showed most effective outcome in respect to 10 mg dose/100 g of body weight. The view for the regenerative activities of this fraction for pancreatic β -cell in STZ-induced diabetes has been reflected here from the recovery in the activities of hexokinase, glucose-6-phosphate dehydrogenase and glucose-6-phosphatase in liver and skeletal muscle as the enzyme hexokinase and glucose-6-phosphate dehydrogenase are regulated by insulin in positive way and glucose-6-phosphatase in negative way [31, 32]. Activities of catalase and SOD were decreased in hepatic and kidney tissues in diabetic condition and recovered significantly by fraction treatment. The said recovery may be due to the antioxidant activity of the phyto molecule (s) present in said extract which protects the catalytic protein molecules in cells from diabetes-induced reactive oxygen species biomolecule injury [33]. This antioxidative activity has been strengthened here from the quantification of TBARS and CD levels in above said tissues as there is an inverse relationship between the activities of antioxidant enzymes and quantity of free radical byproducts which is consistent with other report [34] as well as by our previous works [35, 36]. All these deformities were corrected by the fraction treatment which may be due to recovery in serum insulin [37]. Gene expressions of hexokinase-I in hepatic tissue was assessed to find out the molecular mechanism of the fraction. The positive regulation of hexokinase-I gene may be due to higher level of serum insulin as hexokinase-I expression pattern is modulated by insulin in positive way. In the present study, STZ-induced diabetic animals showed a significant reduction in the gene expression of superoxide dismutase and the genomic activities were significantly recovered after treatment of different doses fraction in comparison to diabetic animals. This remedial effect of the fraction may be due to the stimulation of this fraction on the existing β -cell or regeneration of the β -cell for the recovery in serum insulin and ultimately protecting the cell from apoptosis which was further established by apoptotic markers. Gene expression pattern of Bax and Bcl-2 from diabetic state towards the vehicle treated control may be due to effect of the fraction on the concern gene [38]. After the treatment of different doses of ethyl acetate fraction to diabetic animals it was noted that 20 mg dose is the threshold dose because maximum recovery was observed at this lowest dose [39]. From the comparative analysis of the data, it is observed that 20 mg dose results most promising results in this concern.

CONCLUSION

From the results of this experiment it may be concluded that 20 mg dose of the said fraction of seed of *M. fragrans* is the threshold dose for the management of diabetic disorders with special reference to carbohydrate metabolic and oxidative stress recovery by regeneration of β -cells in pancreas and by stimulating gene expression of carbohydrate metabolic enzymes as well as by recovery of decreasing oxidative stress which may lead to development of herbal drug for such management.

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AUTHORS CONTRIBUTIONS

Ankita Dey performed the research work and wrote the total manuscript. Adrija Tripathy helped to modify a part of the research article. Prof. Debidas Ghosh supervised the entire experiment, result analysis and manuscript correction.

CONFLICT OF INTERESTS

The authors have declared that there is no conflict of interest

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