

Ameliorating effect of mother tincture of *Syzygium jambolanum* on carbohydrate and lipid metabolic disorders in streptozotocin-induced diabetic rat: Homeopathic remedy

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Abstract

Background: *Syzygium jambolanum* (*S jambolanum*) is widely used in homeopathy for treating patients with diabetes mellitus. In the present study, an attempt has been made to investigate the remedial effect of homeopathic drug *S jambolanum* on carbohydrate and lipid metabolic disorders on streptozotocin induced diabetic rat. **Materials and Methods:** Diabetes induction in Wistar strain rat was performed as per standard method using streptozotocin at the dose of 4 mg/100 gm body weight. Activities of carbohydrate metabolic enzymes in hepatic tissue, and glycogen content in hepatic and muscular tissues were assessed biochemically following the standard protocols. Serum lipid profile level and activities of GOT and GPT in serum were measured as per standard method using specific kits. **Results:** The homeopathic drug, mother tincture of *S jambolanum* significantly decreased fasting blood glucose levels and improved carbohydrate metabolic key enzyme activities in hepatic tissue i.e., hexokinase, glucose-6-phosphate dehydrogenase and glucose-6-phosphatase in diabetic rats. Alongside, serum lipid profile biomarkers i.e., triglyceride (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDLc), very low density lipoprotein cholesterol (VLDLc) and high density lipoprotein cholesterol (HDLc) levels were significantly ameliorated in homeopathic drug supplemented diabetic animals in compared with the untreated diabetic animal. Side by side, the *S jambolanum* has the capacity to attenuate diabetes induced hepatic injury in model animal, which has been assessed here by the recovery of GOT and GPT activities in serum of drug treated diabetic animal. **Conclusion:** The result of the present study indicated that the homeopathic drug *S jambolanum* (mother tincture) has a protective effect on diabetic induced carbohydrate and lipid metabolic disorders in STZ-induced diabetic animal.

Key words: Anti-diabetic, antihyperlipidemic, homeopathic remedy, *syzygium jambolanum*

INTRODUCTION

The frequency of diabetes was escalating rapidly worldwide, including developed and developing countries.^[1] In India recent projection indicate that there is an alarming rise in

prevalence of diabetes, which has one beyond epidemic form to a pandemic one.^[2] In modern medicine, there is no satisfactory effective therapy to cure diabetes mellitus.^[3] Synthetic oral hypoglycemic agents can produce a series of side effects including hematological, gastrointestinal reactions, hypoglycemic coma and disturbances in liver and kidney.^[4]

Growing popularity of complementary and alternative medicine (CAM) in the public sector is reflected in the scientific community by an increased number of researches to assessing the efficacy of CAM.^[5] Because this practice may be attributed to the professed

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Quick Response Code: 	Website: www.jnsbm.org
	DOI: 10.4103/0976-9668.107263

outcome, negligible side effects or toxic contribution and comparatively cost effective than synthetic drugs.^[6] Homeopathy is one of the most widespread alternative system of medicine based on the two cardinal principles “law of similarities” and “minimal dilution”.^[7] Homeopathy seeks to cure in accordance with natural laws of healing and uses medicine made from natural substances viz. animal, vegetable and mineral.^[8] Some important homeopathic oral hypoglycaemic drugs are *Rhus aromatica*, *Syzygium jambolanum*, Uranium nitricum and Acid Phos.

In homeopathic Materia Medica, *Syzygium jambolanum* (*S jambolanum*) is described as a most useful remedy against diabetes mellitus. It has an immediate effect to manage the high blood sugar. No other remedy causes so marked degree in the diminution of sugar in the urine.^[9]

Syzygium jambolanum (Family-Myrtaceae; commonly known as ‘jambol fruit’ or ‘jamun’) is common herb found in India, Pakistan, Southern Asia and Brazil.^[10] Mother tincture of *S jambolanum* is widely used by homeopathy practitioners for diabetes management. Mother tincture (θ) is defined as the original tincture prepared with the aid of alcohol, directly from the crude drug. It is the precursor for the preparation of different potencies and the starting point for the production of homeopathic medicines.^[11] The chemical composition of the seed extract has been recently reported by a study. It contains glycoside (Jamboline), tannin, ellagic acid and gallic acid as principal ingredients.^[12]

Over the past few years, there has been an increase in the number of preclinical (*in vitro* and *in vivo*) studies aimed at evaluating the pharmacological activity and efficacy of homeopathic remedies. However, scientific studies on homeopathic remedy regarding its anti-diabetic mechanism of action is scanty.^[13] Therefore, the present study has been conducted to ascertain the pharmacological activity of *S jambolanum* on STZ induced diabetic animal model in connection to explore the anti-diabetic mechanism of action. To fulfil this objective, different relevant biomarkers like carbohydrate metabolic enzyme activities in hepatic tissue, glycogen content in liver and skeletal muscle, serum level of lipid profile biomarkers as well as GOT and GPT activities in serum were assessed.

MATERIALS AND METHODS

Study setting

All the experimental activities were carried out at the Department of Bio-Medical Laboratory Science and Management, Vidyasagar University, Midnapore, West Bengal, India.

Animals

Healthy male Wistar strain albino rats, weighting between 140 and 160 g were used for this study. Rats were housed in our well ventilated animal house having six animals in polypropylene made cages and maintained standard condition with controlled temperature ($25 \pm 3^\circ\text{C}$), relative humidity ($45 \pm 5^\circ\text{C}$) and 12:12 h dark:light cycle. The animals were fed with standard laboratory diet and allowed to drinking water *ad libitum*. Experiments were conducted following the guidelines of our Institutional Animal Ethics Committee (IAEC).

Chemicals

STZ was purchased from Sigma, USA. Other chemicals like adenosine tri phosphate (ATP), nucleotide adenine dinucleotide phosphate (NADP), 2-[4-(2-Hydroxyethyl) 1-piperazinyl] ethanesulfonic acid (HEPES), were purchased from Sigma-Aldrich Diagnostic Ltd. or Sisco Research Laboratory (SRL), India. Kits for the assessment of serum lipid profile level and glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) activities were purchased from Span Diagnostic Ltd. Surat, India.

Induction of diabetes

Rats were made diabetic by a single intramuscular injection of streptozotocin (STZ) at the dose of 40 mg/kg body weight in 0.1 M citrate buffer (pH 4.5).^[3,4] Diabetic state was confirmed on seventh day of STZ injection considering the measurement of FBG as relevant biomarker. Animals with FBG level more than 300 mg/dL were selected for this experiment.

Homeopathic remedy

The homeopathic remedy *S jambolanum* (θ), was purchased from ‘Hahnemann Publishing Company (Hapco)’, 165, Bepin Behari Ganguly Street, Kolkata, India. From this 1 ml of mother tincture of *S jambolanum* was finally diluted with 20 ml of double distilled water to make the stock solution. Each rat were fed 1 drop (0.06 ml) of *S jambolanum* twice a day from the stock solution using gavage. The drug feeding was made before food delivery in subsequent days till they were sacrificed for analysis.

Experimental design

To fulfil the aims of this study, animals were divided into four groups and each groups comprising of 6 rats. Group wise animal distribution was as follows:

Untreated control

Animals of this group were treated with 0.6 ml of diluted ethanol (vehicle) for 40 days at the time of mother tincture treatment to diabetic animals.

Diabetic control

Animals of this group were given vehicle solution for 40 days at the same time.

Diabetic + *S jambolanum*

Animals of this group were treated with 0.06 ml of mother tincture of *S jambolanum* for 40 days.

The duration of the experiment was 40 days. On forty-first day, all the animals were sacrificed by decapitation. Blood was collected from dorsal aorta by a syringe, the serum was separated by centrifugation at 3000 g for 10 mins for the measurements of serum lipid profile level and activities of GOT and GPT in serum. The liver, kidney and skeletal muscle were dissected out and stored at -20°C for biochemical analysis of the activities of carbohydrate metabolic enzymes in hepatic tissue and for the quantification of glycogen in the concern hepatic and skeletal tissue.

EXPERIMENTAL PARAMETERS

Fasting blood glucose level

Fasting blood glucose level in animals of all the groups were measured as per our reported method^[3] by single touch glucometer (Bayer's Ascensia Entrust, Bayer, and Germany) on every tenth day.

Carbohydrate metabolic enzyme activities

Activities of carbohydrate metabolic key enzymes i.e., hexokinase,^[15] glucose-6-phosphate dehydrogenase^[16] and glucose-6-phosphatase^[17] in hepatic tissue were measured biochemically by recording OD using spectrophotometer.

Glycogen content

Glycogen levels in liver and skeletal muscle were measured according to the standard method.^[18]

Measurement of lipid profile

Serum lipid profile like serum levels of triglycerides (TG),^[19] total cholesterol (TC),^[20] low density lipoprotein cholesterol (LDLc),^[21] very low density lipoprotein cholesterol (VLDLc)^[22] and high density lipoprotein cholesterol (HDLc)^[22] were estimated biochemically using specific kits in this concern.

Biochemical assay of glutamate oxaloacetate transaminase, glutamate pyruvate transaminase activities in serum

Activities of GOT and GPT in serum were measured according to the standard method using specific kits in this concern.^[23]

Data analysis

All the data were represented as Mean \pm SEM ($n = 6$). Analysis of the data was carried out by one-way analysis of variance (ANOVA) followed by multiple comparisons two tail 't' test using statistical software (Origin version 8.1). P values < 0.05 were considered as statistically significant.

RESULTS

Fasting blood glucose level

STZ induced diabetic animals resulted in a significant elevation ($P < 0.05$) in fasting blood glucose levels (>300 mg/dL) in respect to untreated control animals. After the treatment of homeopathic remedy *S jambolanum* to diabetic animals for 40 days, a significant reduction ($P < 0.05$) of fasting blood glucose level was noted in compare with untreated diabetic animals. During experimental period, on tenth day by 19.1%, twentieth day by 47.2%, thirtieth day by 60.0% and fortieth day by 69.4% reduction of fasting blood glucose level were observed in mother tincture of *S jambolanum* treated diabetic group which focused the antihyperglycemic efficacy of *S jambolanum* in STZ-induced diabetic animals [Figure 1].

Carbohydrate metabolic enzyme activities

STZ induced diabetic animals resulted in a significant diminution ($P < 0.05$) in the activities of hexokinase and glucose-6-phosphate dehydrogenase along with significant elevation ($P < 0.05$) in glucose-6-phosphatase activity in hepatic tissue in respect to the untreated control. After treatment of the said drug to diabetic rats there was a significant recovery ($P < 0.05$) in the activities of hexokinase, glucose-6-phosphatase and glucose-6-phosphate dehydrogenase in respect to diabetic control [Figures 2-4].

Serum lipid profile

Levels of serum TG, TC, LDLc and VLDLc were increased significantly ($P < 0.05$) in STZ-induced diabetic group in respect to untreated control. After administration of the homeopathic drug to diabetic group of animals, a significant recovery ($P < 0.05$) was noted towards the control. In homeopathic drug treated diabetic group of animals, serum HDLc level was resettled towards the control level significantly ($P < 0.05$) [Table 1].

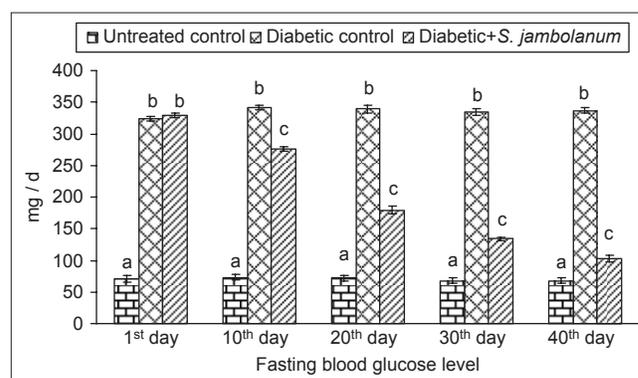


Figure 1: Remedial effect of mother tincture of *S jambolanum* on blood glucose level in STZ-induced diabetic rats. Bars represent Mean \pm SEM ($n = 6$). Bars with superscripts a, b, c differ from each other significantly at $P < 0.05$

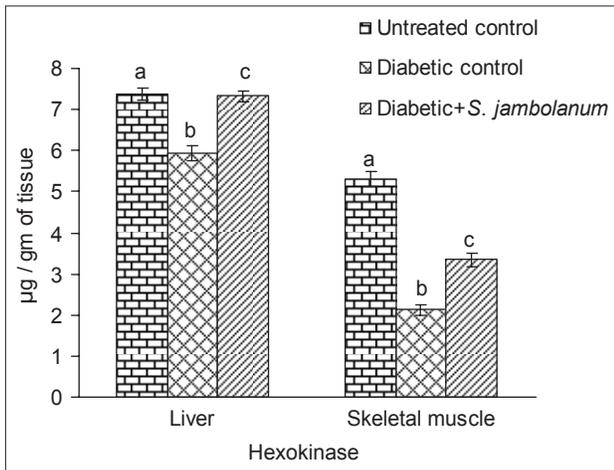


Figure 2: Remedial effect of mother tincture of *S. jambolanum* on hepatic and skeletal muscular hexokinase activity in STZ-induced diabetic rats. Bars represent mean \pm S.E.M. ($n = 6$). Bars with superscripts a, b, c differ from each other significantly at $P < 0.05$

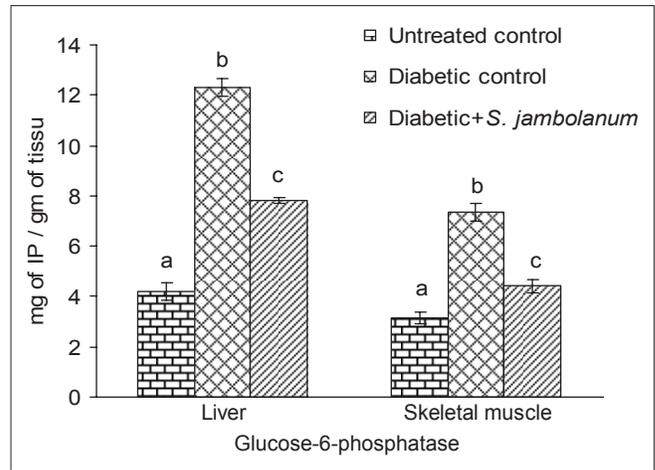


Figure 3: Protective effect of mother tincture of *S. jambolanum* on hepatic and skeletal muscular glucose-6-phosphate activity in STZ-induced diabetic rats. Bars represent mean \pm S.E.M. ($n = 6$). Bars with superscripts a, b, c differ from each other significantly at $P < 0.05$

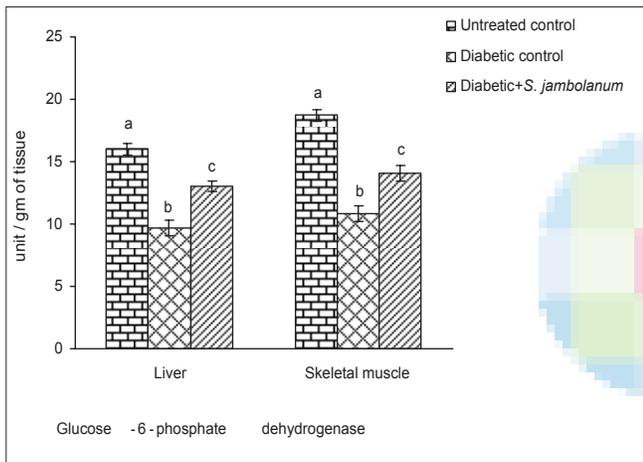


Figure 4: Activities of Glucose-6-phosphate dehydrogenase in liver and skeletal muscle in STZ-induced diabetic rats after the treatment of *S. jambolanum*. Bars represent mean \pm S.E.M. ($n = 6$). Bars with superscripts a, b, c differ from each other significantly at $P < 0.05$

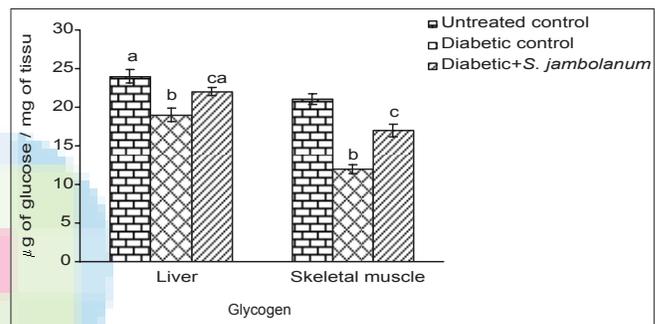


Figure 5: Glycogen level in metabolic tissues (liver and skeletal muscle) in STZ-induced diabetic rats. Bars represent Mean \pm SEM ($n = 6$). Bars with superscripts a, b, c differ from each other significantly, $P < 0.05$

Glycogen content

Quantity of glycogen in liver and skeletal muscle which was decreased significantly ($P < 0.05$) in diabetic group of animals in respect to untreated control was recovered significantly ($P < 0.05$) after the drug treatment to diabetic animals [Figure 5].

Levels of glutamate oxaloacetate transaminase, glutamate pyruvate transaminase activities in serum

Activities of GOT, GPT in serum were increased significantly ($P < 0.05$) in diabetic control group in respect to untreated control group of animals. Significant recoveries ($P < 0.05$) were observed in the activities of serum GOT and GPT in drug treated diabetic group when comparison was made with diabetic control group [Figure 6].

DISCUSSION

STZ-induced diabetes causes the partial destruction of β -cell of the islets which leads to insufficient release of

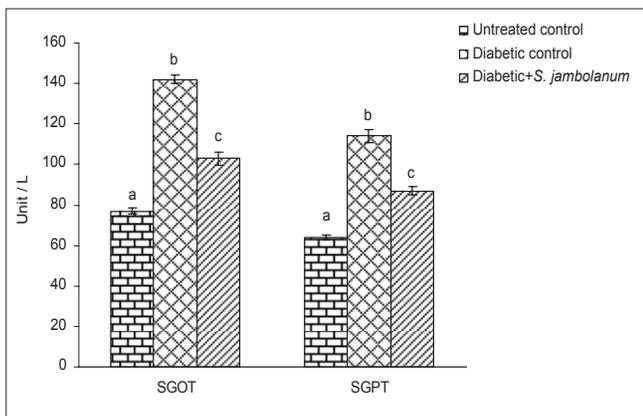


Figure 6: Levels of serum GOT, GPT activities in STZ-induced diabetic rats. Bars represent Mean \pm SEM ($n = 6$). Bars with superscripts a, b, c differ from each other significantly, $P < 0.05$

Table 1: Effect of mother tincture of *S jambolanum* on serum lipid profiles in STZ-induced diabetic rats

Groups	Lipid profile levels (mg/dL)				
	TG	TC	HDLc	LDLc	VLDLc
Untreated control	60±1.4 ^a	52±1.7 ^a	24±1.3 ^a	19±1.5 ^a	12±0.8 ^a
Diabetic control	125±2.2 ^b	97±2.1 ^b	11±1.7 ^b	39±1.7 ^b	25±1.4 ^b
Diabetic+ <i>S jambolanum</i>	70±1.7 ^c	72±1.8 ^c	17±1.4 ^c	26±1.4 ^c	14±0.8 ^c

Values represent Mean ± SEM (n=6). Values with superscripts ^{a,b,c} in vertical column differ from each other significantly, P<0.05, TG=Triglyceride, TC=Total cholesterol, HDL=High density lipoprotein cholesterol, LDL=Low density lipoprotein cholesterol, VLDL=Very low density lipoprotein cholesterol

insulin and there by increased blood glucose levels namely hyperglycemia.^[24] Administration of homeopathic remedy *S jambolanum* to diabetic animals significantly reduced the elevated blood glucose level, may be due to the stimulatory effect of *S jambolanum* on remaining β-cells of the islets of Langerhans to produce insulin or regeneration of pancreatic β-cells, which is concur with other report in this line.^[25]

The corrective effect of the *S jambolanum* was observed from the assessment of the activities of hepatic hexokinase, glucose-6-phosphate dehydrogenase those are significantly increased in mother tincture treated diabetic group, indicate the insulinotropic effect as these enzymes are regulated positively by insulin.^[3] Significant decrease in the activity of hepatic glucose-6-phosphatase by this drug indicates insulinotropic effect of the drug as this enzyme is regulated negatively by insulin.^[26] This result was supported from the increased levels of glycogen in liver and skeletal muscle in mother tincture treated group.

The levels of serum lipid profile are usually elevated in diabetes mellitus and such an elevation represents the risk of coronary heart disease.^[26] In the STZ-induced diabetes, the rise in blood glucose is accompanied by an increase in various blood lipids those are TG, TC, LDLc, VLDLc and HDLc levels. Treatment of *S jambolanum* recovered the blood lipids significantly which indicate its antihyperlipidemic efficacy. The effect of *S jambolanum* on diabetic hyperlipidemia may be due to the control of hyperglycemia, which is coincide with our previous reports^[27,28] and by others.^[29]

Activities of pathophysiological enzymes such as SGOT and SGPT which are hepatic marker enzymes, have leaked into the circulation during hepatocyte injury.^[30] Hence, the observed increase in the activities of GOT and GPT in serum of diabetic rats may primarily be due to leakage of these enzymes from liver cytosol into bloodstream as a consequence of hepatic injury by STZ as proposed by other.^[31] Oral administration of *S jambolanum* to diabetic rats significantly decreased the activities of these enzymes, suggesting the hepatoprotective nature of *S jambolanum* mother tincture in experimentally induced diabetic hepatic injury and this observation is supported by other researchers.^[32]

There was a clear evidence from this study that homeopathic drug *S jambolanum* indeed positively protective effects on STZ induced diabetic rats. Recently, an elegant study demonstrated that ethanolic extract of *S jambolanum* has a great potential in therapeutic use as anti-diabetic drug.^[33]

However, to explore the exact mechanism of action of *S jambolanum* on experimentally induced diabetes, more work is required in this line covering the molecular biological biosensors, which are presently underway. Finally, the present study convincingly demonstrated an ameliorative effect of mother tincture of *S jambolanum* on diabetic complication in STZ-induced diabetic animals.

ACKNOWLEDGMENTS

The authors are indebted to Dr. Shyamapada Paul for the inspiration in this research work. We are gratefully acknowledged to Purusottam Homeo Pharmacy, Midnapore for their cooperation.

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How to cite this article: Maiti S, Ali KM, Jana K, Chatterjee K, De D, Ghosh D. Ameliorating effect of mother tincture of *Syzygium jambolanum* on carbohydrate and lipid metabolic disorders in streptozotocin-induced diabetic rat: Homeopathic remedy. *J Nat Sc Biol Med* 2013;4:68-73.

Source of Support: Nil. **Conflict of Interest:** None declared.

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