

ORIGINAL ARTICLE

A study of the effect of mother tincture of *Syzygium jambolanum* on metabolic disorders of Streptozotocin induced diabetic male albino rat

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ABSTRACT

Background: *Syzygium jambolanum* is widely used in Homoeopathy for treating Diabetes mellitus and its complications.

Objective: To explore the remedial effects of homoeopathic mother tincture *Syzygium jambolanum* on metabolic disorders of Streptozotocin induced diabetic male albino rat.

Materials and Methods: Serum levels of insulin, urea, uric acid, creatinine, albumin and total protein were measured as per the standard methods using specific kits. Amount of glycogen in liver and skeletal muscles, activities of antioxidant enzymes as well as content of free radical by products in liver and kidney tissues were assessed biochemically following the standard protocol.

Results: The study indicated that the treatment of mother tincture of *Syzygium jambolanum* in diabetic albino rats restored the body weight and significantly controlled the elevated blood glucose level as compared with the untreated group. Levels of glycogen in liver and skeletal muscle tissues were recovered by treatment with *Syzygium jambolanum* in diabetic rats as compared with the untreated diabetic rats. Levels of serum urea, uric acid and creatinine were increased in diabetic rats significantly as compared with the control group, which were resettled in the control group after treatment with mother tincture of *Syzygium jambolanum* in diabetic animals. Alongside, significant recovery in the activities of antioxidant enzymes like catalase, peroxidase and super oxide dismutase, the levels of free radicals generated as by products in hepatic and renal tissues were also observed in the treatment with mother tincture of *Syzygium jambolanum* treated diabetic animals with respect to the untreated in diabetic animals.

Conclusion: The homoeopathic mother tincture of *Syzygium jambolanum* has therapeutic effect on metabolic disorders and oxidative injuries in Streptozotocin induced diabetic male albino rats.

Keywords: Antidiabetic, Antioxidant, Diabetes, Homeopathy, Oxidative Stress Markers, Streptozotocin

INTRODUCTION

Homoeopathy, a common form of alternative medicine is used worldwide and plays a major role in healing

different diseases.^[1] Due to minimal side-effects, homoeopathic remedies may serve as potential method of treatment and in the management of diabetes.^[2]

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The incidence of diabetes is rapidly increasing, particularly in the developing countries due to urbanisation, genetic predisposition and lifestyle.^[3,4] It has a considerable impact on the health, life style, life expectancy of patients and its complications result in major health problems.^[5] It is a metabolic disease, characterized by hyperglycemia, impaired metabolism of glucose and other energy-yielding fuels, such as lipids and proteins.^[6] This metabolic disorder is the result of a deficiency in insulin secretion or resistance to insulin action, or both.^[7]

Chronic hyperglycaemia in diabetes mellitus induces multiple bio-chemical sequelae including diabetes-induced oxidative stress which plays a vital role in the symptoms and progression of the disease.^[8] Oxidative stress in cells and tissues results in increased generation of Reactive Oxygen Species (ROS) from decrease in antioxidant defense potential.^[9] Several hypothesis like auto-oxidation process of glucose, the non-enzymatic and progressive glycation of proteins with the consequent increased formation of glucose-derived Advanced Glycosylated End products (AGEs), and enhanced glucose flux through the polyol pathway have been put forth to explain the genesis of free radicals in diabetes.^[10] Generation of free radicals results in consumption of antioxidant defense components leading to cellular dysfunction and hence triggers cellular death.^[11]

Syzygium jambolanum, traditional homoeopathic remedy is used clinically to treat people with diabetes. It is reported to have an effect in managing the high blood sugar.^[12] Scientific investigations have been reported on the treatment with mother tincture of *Syzygium jambolanum* regarding antihyperglycemic activity^[13] antihyperlipidemic activity^[14] and antidiabetic activity^[15] in animal model. No study has been designed to find out its effect on oxidative stress status and metabolic disorders in diabetic animal. The present study has been conceived to search the effect of mother tincture of *Syzygium jambolanum* on oxidative stress markers in diabetic animals along with glycaemic and protein metabolic bio-sensors.

MATERIALS AND METHODS

Animal and Animal Care

Wistar strain albino male rats weighing 140–180 gm were used in the present study. All animals were

housed in clean polypropylene cages and maintained under standard laboratory conditions (temperature $25 \pm 2^\circ\text{C}$ with 12 hour dark: 12 hour light cycle). The standard laboratory diet was provided to the animals and they were allowed to drink water *ad libitum*. The animals were acclimatized to laboratory conditions for 15 days prior to the experiment. Studies were carried out after the approval and following the guidelines of the Institutional Animal Ethics Committee (IAEC), Vidyasagar University, Midnapore, India.

Chemicals

Streptozotocin (STZ) was purchased from Sigma Chemical Co., St. Louis, MO, USA. Trichloroacetic acid (TCA) and thiobarbituric acid (TBA) were purchased from SRL, Mumbai, India. All the other reagents used were of analytical grade obtained commercially.

Homoeopathic Remedy

Homoeopathic mother tincture (Q) of *Syzygium jambolanum* was procured from Hahnemann Publishing Company (HAPCO), Kolkata.^[16] 1ml of *Syzygium jambolanum* mother tincture was diluted with 20 ml of double distilled water to make the stock solution. Each rat was fed with 1 drop (0.06 ml) of *Syzygium jambolanum* twice a day from the stock solution by gavages feeding before 30 minutes of the food intake. The drug feeding continued till the animals were sacrificed for analysis.

Induction of Diabetes

Diabetes mellitus was induced on overnight fasted rats by administering single intramuscular injection of freshly prepared STZ in 0.1 M citrate buffer (pH - 4.5) at the dose of 4 mg/100 gm body weight.^[17] Diabetes was detected 72 hour from fasting blood glucose levels. Diabetic rats were kept 7 days under standard laboratory condition for the stabilisation of hypoglycaemic state. After 7 days, rats with blood glucose 300gm/dL were selected for the study.

Experimental Design

The albino rats were divided into four groups with six rats in each group as follows:

a. Control Group

Normoglycaemic rats who received 0.06 ml of diluted ethanol (1:20) through oral route as vehicle treatment for 40 days during the trial.

b. Diabetic Group

STZ-induced diabetic rats who were treated

with 0.06 ml of diluted ethanol through out the experiment as vehicle treatment.

c. Diabetic + *S. jambolanum* Group

Diabetic rats who were treated with 0.06 ml/100 gm body weight/rat of mother tincture of *Syzygium jambolanum* for 40 days.

d. Diabetic + Glibenclamide Group

Diabetic rats who were treated with Glibenclamide at the dose of 0.06 mg dissolved in 0.06 ml of water/100 gm body weight/rat for 40 days 18.

The vehicle or *Syzygium jambolanum* or Glibenclamide was administered orally to the respective group of animals for 40 days. Fasting blood glucose levels in all the groups were monitored using single touch Glucometer on every 10th day. On the 41st day of the experiment (considering the day of mother tincture or Glibenclamide treatment as the 1st day), all the animals were sacrificed by decapitation. Blood was collected from dorsal aorta and serum was separated by centrifugation for the measurement of serum levels of insulin, urea, uric acid and creatinine. Liver, kidney and skeletal muscle tissues were dissected out and preserved for biochemical analysis of the antioxidant enzyme activities and the quantification of glycogen and free-radical by-products in respective tissue sample.

Fasting blood glucose level and body weight

Fasting blood glucose level was measured at the time of STZ injection. After 7 days, the fasting blood glucose levels of all the rats were measured at the interval of 10 days i.e. (10th, 20th, 30th and 40th day). The blood glucose was estimated by single touch Glucometer (Bayer's Ascensia Entrust, Bayer, Germany). Initial and final body weights of all the rats from each group were recorded by digital weighing machine.

Glycogen level

Glycogen level of liver and skeletal muscle tissues were measured according to the standard methods^[19] with a slight modification.^[20] The results were expressed in terms of μg of glucose/mg of tissue.

Serum biochemical parameters

Serum insulin level was measured using solid phase conjugated sandwich ELISA kit for rat (EZRMI-13K, Millipore, USA).^[21] The optical density of standard and unknown samples were measured against blank using 480 nm selective filter and 650 nm

differentiating filter. Serum urea, uric acid, creatinine and albumin were estimated by spectrophotometric method using specific kits of Merck Diagnostic Ltd, India.^[22]

Oxidative stress related biomarkers

The catalase enzyme activities of hepatic and renal tissues were measured biochemically.^[23] For the evaluation of catalase activities, target samples were homogenized separately in 0.05 M Tris-HCl buffer solution (pH:7.0) at a tissue concentration of 50 mg/mL. These homogenized solutions were centrifuged at 10,000g at 4°C for 10 minutes. Mixture of 0.5 mL of 35 mM H₂O₂ and 2.5 mL of distilled water were mixed and transferred to a spectrophotometric cuvette. The absorbance was measured at 240 nm. Sample supernatant of 40 μL was added and subsequent six readings were noted at 30 seconds interval.

Activities of peroxidase (Px) enzyme of target tissues were measured according to the standard method.^[24] Guaiacol (20mM) was mixed with 0.1 mL of sample. In presence of 0.3 mL of 12.3 mM H₂O₂, the time was recorded for an increase in the absorbance by 0.1 at 436 nm.

The superoxide dismutase (SOD) enzyme activities of the tissue samples were estimated by measuring the percentage inhibition of the pyrogallol auto oxidation by SOD according to the standard method.^[25] The buffer was prepared by 50 mM Tris (pH 8.2). In a spectrophotometric cuvette, 2.04 mL of TRIS buffer, 20 mL of sample and 20 mL of pyrogallol were taken and the absorbance was noted in spectrophotometer at 420 nm for 3 min period. One unit of SOD was defined as the enzyme activity that inhibits the auto-oxidation of pyrogallol by 50%.

Estimation of end-products of lipid peroxidation (TBARS)

The sample tissues were homogenized separately at the tissue concentration of 50 mg/mL in 0.1 M of ice-cold phosphate buffer (pH 7.4) and the homogenates were centrifuged at 10,000 g at 4°C for 5 minutes separately. Each supernatant was used for the spectrophotometric quantification of Thiobarbituric acid reactive substances (TBARS) following standard method.^[26]

Statistical analysis

Experimental data were expressed as Mean \pm SEM. Statistical significance was analyzed by one-way

analysis of variance (ANOVA) followed by two tail 't' test using Origin statistical software (version 8.1) and P value < 0.05 was considered as statistically significant.

RESULTS

Fasting blood glucose levels

The fasting blood glucose (FBG) levels of control, diabetic and *Syzygium jambolanum* treated groups are in Figure 1. The STZ at the dose of 4 mg/100 gm body weight resulted in marked hyperglycemia as evident from significant elevation ($P < 0.05$) in FBG level in untreated diabetic animals in comparison with the control group. The diabetic+ *Syzygium jambolanum* group or diabetic+ Glibenclamide group rats showed a significant reduction ($P < 0.05$) in fasting blood glucose levels as compared to control group. Treatment of diabetic rats with Glibenclamide showed definite variation in the level of difference in glucose level in comparison with *Syzygium jambolanum* treated diabetic rats [Figure 1].

Body weight, glycogen and plasma insulin levels

The changes in body weight, glycogen content in liver and skeletal muscles and plasma insulin levels of control, diabetic, Glibenclamide and *Syzygium jambolanum* treated rats is described in Table 1. Administration of mother tincture of *Syzygium jambolanum* for 40 days to diabetic animals significantly corrected ($P < 0.05$) body weight reduction which was reduced due to STZ injection ($P < 0.05$) in diabetic rats, liver and muscle glycogen levels were significantly decreased than control rats ($P < 0.05$).

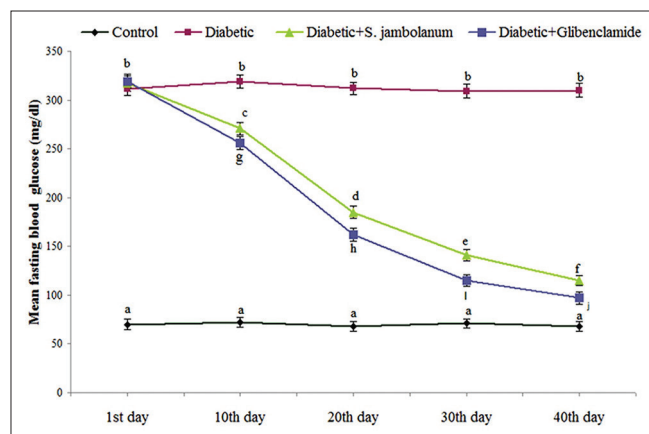


Figure 1: Effects of mother tincture *Syzygium jambolanum* on fasting blood glucose level in the STZ-induced diabetic rat

Both *Syzygium jambolanum* and Glibenclamide treated diabetic groups showed an increase ($P < 0.05$) in liver and muscle glycogen content in respect to untreated diabetic rats. The plasma insulin level was reduced ($P < 0.05$) in the diabetic group, where as treatment of diabetic rats with *Syzygium jambolanum* or Glibenclamide showed increased ($P < 0.05$) insulin level when comparison was made with untreated diabetic rats. In all the above parameters, Glibenclamide was found to be effective ($P < 0.05$) than *Syzygium jambolanum* treated diabetic animals.

Protein metabolism markers

The result of protein metabolism bio-sensors in diabetic, Glibenclamide and *Syzygium jambolanum* treated rats is shown in Table 2. Serum urea, uric acid and creatinine levels were found to be increased ($P < 0.05$) and the albumin levels were decreased ($P < 0.05$) in diabetic rats than in control group. Administration of *Syzygium jambolanum* or Glibenclamide for consecutive 40 days on diabetic rat reduced these parameters ($P < 0.05$) as compared to the controlled group. The significant ($P < 0.05$) recovery was observed in diabetic -Glibenclamide group with respect to the diabetic - *Syzygium jambolanum* group.

Oxidative stress biomarkers

After 40 days treatment with *Syzygium jambolanum* or Glibenclamide to the diabetic rats, a significant elevation ($P < 0.05$) in oxidative stress biomarkers were observed. But recovery was also significant in diabetic -Glibenclamide group when compared with diabetic - *Syzygium jambolanum* group [Table 3]. CAT, SOD and Px significantly ($P < 0.05$) reduced in diabetic -Glibenclamide compared with the control group.

Thiobarbituric Acid Reactive Substances (TBARS) levels were significantly increased ($P < 0.05$) in diabetic rats compared to the control rats. Diabetic rats treated with *Syzygium jambolanum* or Glibenclamide showed significant ($P < 0.05$) decrease in TBARS levels in the liver and kidney tissues. A significant variation was observed in this level of the sensors between *Syzygium jambolanum* treated and Glibenclamide-treated groups Figure 2.

DISCUSSION

The STZ-induced diabetes is associated with the generation of reactive oxygen species causing

oxidative damage^[26] and destruction of β -cells, developing an insulin-dependent diabetes mellitus and weight loss.^[28]

In the present study, treatment with mother tincture of *Syzygium jambolanum* in diabetic rats showed reduction in the fasting blood glucose levels which confirms the antihyperglycaemic effect of homoeopathic preparation of *Syzygium jambolanum*.^[13,14] Experimental studies show that STZ-induced diabetic rats lose their body weight which may be due to dehydration and the catabolism of body fats and proteins.^[29] This results of this study show that administration of *Syzygium jambolanum* partially improved the body weight in diabetic rats, which further supports therapeutic effect of the drug.

Both the glycogen content of liver and skeletal muscles was reduced in STZ-induced diabetic

rats. The lack of insulin causes a decrease in the glycogen content in the diabetic state, which results

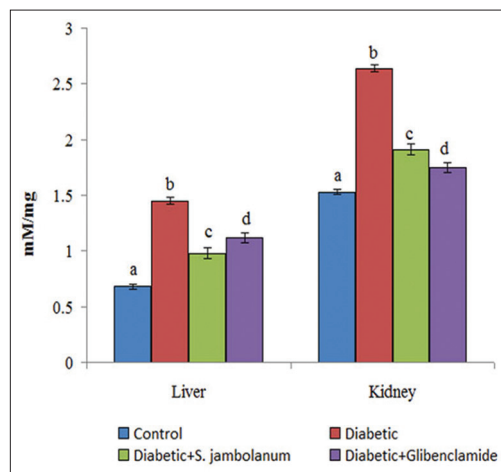


Figure 2: Effect of mother tincture of *Syzygium jambolanum* on Thiobarbituric Acid Reactive Substances (TBARS) level in the STZ-induced diabetic rat

Table 1: Effects of mother tincture of *S. jambolanum* on body weight, glycogen content and plasma insulin level in the STZ-induced diabetic rat

Groups	Body weight (g)		Glycogen (μ g of glucose/mg of tissue)		Plasma insulin (μ U/mL)
	Initial	Final	Liver	Skeletal muscle	
Control	149 \pm 2.5 ^a	157 \pm 2.2 ^a	46.23 \pm 1.20 ^a	38.9 \pm 0.98 ^a	15.8 \pm 0.65 ^a
Diabetic	150 \pm 2.1 ^a	139 \pm 2.5 ^b	27.43 \pm 1.44 ^b	23.17 \pm 1.44 ^b	5.98 \pm 0.54 ^b
Diabetic+ <i>Syzygium jambolanum</i>	148.5 \pm 3.7 ^a	155 \pm 3.2 ^a	35.21 \pm 1.67 ^c	31.81 \pm 1.33 ^c	9.1 \pm 0.83 ^c
Diabetic+Glibenclamide	148 \pm 2.2 ^a	153 \pm 2.9 ^a	39.63 \pm 1.74 ^c	34.7 \pm 1.61 ^c	10.8 \pm 0.37 ^d

Data are represented as mean \pm SEM (n=6). ANOVA followed by multiple comparison two tail 't' test. Values with superscripts (a, b, c, d) in each vertical column differ from each other significantly, P<0.05

Table 2: Effects of mother tincture of *Syzygium jambolanum* on serum levels of urea, uric acid, creatinine and albumin in the STZ-induced diabetic rat

Groups	Urea (mg/dL)	Uric acid (mg/dL)	Creatinine (mg/dL)	Albumin (mg/dL)
Control	35.4 \pm 1.24 ^a	4.3 \pm 0.65 ^a	0.59 \pm 0.12 ^a	4.6 \pm 0.68 ^a
Diabetic	69.3 \pm 1.33 ^b	11.6 \pm 1.3 ^b	1.7 \pm 0.14 ^b	9.1 \pm 0.55 ^b
Diabetic+ <i>Syzygium jambolanum</i>	49.5 \pm 0.93 ^c	7.98 \pm 0.93 ^c	1.01 \pm 0.09 ^c	7.4 \pm 0.78 ^c
Diabetic+Glibenclamide	45.7 \pm 1.12 ^d	6.6 \pm 0.89 ^d	0.89 \pm 0.12 ^d	7.4 \pm 1.1 ^c

Data are represented as mean \pm SEM (n=6). ANOVA followed by multiple comparison two tail 't' test. Values with superscripts (a, b, c, d) in each vertical column differ from each other significantly, P<0.05

Table 3: Activities of antioxidant enzymes (CAT, SOD, Px) in liver and kidney tissues of control, diabetic and *S. jambolanum* in the STZ-induced diabetic rat

Groups	CAT (μ M H ₂ O ₂ consumed/mg of tissue/min)		Px (unit/mg of tissue)		SOD (unit/mg of tissue/min)	
	Liver	Kidney	Liver	Kidney	Liver	Kidney
Control	9.21 \pm 0.23 ^a	8.7 \pm 0.28 ^a	3.7 \pm 0.19 ^a	3.1 \pm 0.22 ^a	5.8 \pm 0.26 ^a	6.9 \pm 0.32 ^a
Diabetic	3.8 \pm 0.21 ^b	3.4 \pm 0.26 ^b	2.0 \pm 0.27 ^b	1.8 \pm 0.24 ^b	2.4 \pm 0.31 ^b	2.6 \pm 0.28 ^b
Diabetic+ <i>Syzygium jambolanum</i>	5.1 \pm 0.19 ^c	5.7 \pm 0.31 ^c	2.5 \pm 0.18 ^c	2.1 \pm 0.25 ^c	4.8 \pm 0.29 ^c	4.7 \pm 0.33 ^c
Diabetic+Glibenclamide	6.4 \pm 0.24 ^d	5.9 \pm 0.30 ^d	3.1 \pm 0.19 ^d	2.6 \pm 0.21 ^d	5.2 \pm 0.31 ^d	5.8 \pm 0.24 ^d

Data are represented as mean \pm SEM (n=6). ANOVA followed by multiple comparison two tail 't' test. Values with superscripts (a, b, c, d) in each vertical column differ from each other significantly, P<0.05

in inactivation of the glycogen synthase system.^[30] Administration of *Syzygium jambolanum* significantly improved the liver and muscle glycogen levels in diabetic rats, possibly due to the reactivation of the glycogen synthase system as a result of increased insulin secretion and thereby elevation of glycogen content.^[8,13,15,30]

Serum urea, uric acid and creatinine levels were significantly increased in diabetic group compared to that of control due to excessive breakdown of body protein.^[31] The treatment with *Syzygium jambolanum* decreased the levels of serum urea, uric acid, albumin level and creatinine due to the improvement of carbohydrate metabolism, thereby reducing the breakdown of body protein.

Oxidative stress in diabetes has been shown to coexist with impairment in the endogenous antioxidant status.^[32] The reduction of hepatic and renal CAT, Px and SOD activities in STZ-induced diabetic rats in this study is also consistent with others.^[32] Here, the results indicate that treatment with *Syzygium jambolanum* restored the parameters of CAT, Px and SOD in liver and kidney. From these results, it may be assumed that due to antihyperglycaemic effects of *Syzygium jambolanum* there is reduction of hypoglycaemia-induced ROS generation and ultimately diabetes induced oxidative injury. The results confirm improvement in TBARS levels in diabetic - *Syzygium jambolanum* group compared with diabetic control as there is an inverse relationship between antioxidant enzyme activities and TBARS levels.^[32]

Overall results indicate that the treatment with mother tincture of *Syzygium jambolanum* has therapeutic effects in managing diabetic disorders like oxidative stress, disorders of protein metabolism and in the correction of hyperglycaemia in STZ-induced diabetes. So, this homeopathic remedy may serve as potential medicine for the management of diabetes and its complications.

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स्ट्रेप्टोजोटोसिन प्रेरित मधुमेह अवर्णी चूहों में उपापचयी व्याधियों में साइजीजियम जम्बोलेनम (जामुन) के प्रभाव के लिए अध्ययन: एक होम्योपैथिक पहुँच सार:

उद्देश्य: स्ट्रेप्टोजोटोसिन प्रेरित मधुमेह अवर्णी चूहों में उपापचयी व्याधियों में साइजीजियम जम्बोलेनम मदर टिंचर के उन्नत करने वाले प्रभाव का पता लगाने के लिये।

सामग्री एवं विधियाँ: मानक विधियों के अनुसार विशेष किटो का प्रयोग कर इन्सुलिन के सीरम स्तर, यूरिया, यूरिक एसिड, क्रियेटिनिन, एल्बुमिन और कुल प्रोटीन को मापा गया। मानक प्रोटोकॉल के अनुसार कंकालीय मांसपेशियों एवं यकृत में ग्लाइकोजन की मात्रा, प्रति ऑक्सीकारक एजाइमों की क्रिया विधि और यकृत एवं गुर्दा उत्तक के उत्पाद द्वारा स्वतन्त्र कणों का जैव रासायनिकीय मूल्यांकन किया गया।

परिणाम: परिणाम यह प्रदर्शित करता है कि अवर्णी मधुमेही चूहों में साइजीजियम जम्बोलेनम के मदर टिंचर (अर्क) के उपचार ने भार को पूर्णसंचित किया व उच्चशर्करा (ग्लूकोज) के स्तर को भी महत्वपूर्ण रूप से बिना उपचारित अवर्णी चूहों की तुलना में अधिक नियन्त्रित किया। यकृत और कंकालीय मांसपेशियों में भी ग्लूकोज के स्तर (पी <0.05) को बिना उपचारित अवर्णी चूहों की तुलना में अधिक पुनःसमरण किया। इन्सुलिन के स्तर यूरिया, यूरिक एसिड, क्रियेटिनिन, एल्बुमिन और कुल प्रोटीन की मात्रा भी बिना उपचारित जानवरों की तुलना (पी <0.05) में वृद्धि पाई गयी। साथ में बिना उपचारित अवर्णी चूहों की तुलना में साइजीजियम जम्बोलेनम मदर टिंचर उपचारित चूहों में प्रति ऑक्सीकारक एन्जाइम की गतिविधियों जैसे केटालेस, परॉक्सीडेस, एवं सुपर आक्साइड डाइस्मूस्टेज में महत्वपूर्ण सुधार देखा (पी <0.05), यकृत एवं गुर्दे में उत्पन्न मुका कणों के स्तर में भी सुधार किया गया।

निष्कर्ष: स्ट्रेप्टोजोटोसिन प्रेरित मधुमेह अवर्णी नर चूहों में साइजीजियम जम्बोलेनम मदर टिंचर के प्रयोग में आक्सीकारक चोटों एवं उपापचयी व्याधियों में उपचारात्मक प्रभाव रखना है।