Antidiabetic Effects of Separate and Composite Extract of Seed of Jamun 
(Eugenia jambolana) and Root of Kadali (Musa paradisiaca) in 
Streptozotocin-induced Diabetic Male Albino Rat: A Comparative Study

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Abstract: The present study investigates separate as well as composite methanolic extracts of seed of Jamun (Eugenia jambolana) and root of Kadali (Musa paradisiaca) for the management of streptozotocin-induced diabetes in rat. As oxidative stress is one of the consequences of diabetes so the present study have measured the activities of some important carbohydrate metabolic enzymes and oxidative stress condition in important visceral organs. After treatment of methanolic extract of above plant parts in separate or in composite manner in streptozotocin-induced diabetic rat resulted a significant recovery in the activities of hexokinase, glucose-6-phosphatase and glucose-6-phosphate dehydrogenase in liver along with correction in fasting blood glucose as well as liver and skeletal muscle glycogen level and plasma insulin level in respect to diabetic group. Activities of catalase and glutathione peroxidase along with the quantity of thiobarbituric acid reactive substance and conjugated dienes in liver and skeletal muscle were also corrected by these plant extracts in respect to diabetic state. The composite extract shows a significant recovery in parameters mentioned earlier than the separate extract. None of the extract has any metabolic toxicity induction in general. From this experiment it may be concluded that the composite extracts of above plants parts have some potential antidiabeticogenic activities.

Key words: Eugenia jambolana, Musa paradisiaca, diabetes, oxidative stress

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion or action or both (Georg and Ludvik, 2000; Nyholm et al., 2000). It is associated with serious complications like polyuria, polyphagia, polydypsia, ketosis, nephropathy, neuropathy and cardiovascular disorders (Gandjbakhch et al., 2005) and at present it is known as syndrome (Zimmet, 1997). There is increasing evidence that complications related to diabetes are associated with oxidative stress induced by generation of free radicals (Armstrong and Al-Awadi, 1991). Several studies showed that antioxidant treatment reduces diabetic complications (Wohabe and Godin, 1987; Simon and Eriksson, 1997). In modern medicine no satisfactory effective therapy is still available to cure diabetes mellitus. Though insulin therapy is used for management of diabetes mellitus but there are several drawbacks which include insulin allergy, insulin antibodies, lipodystrophy, auto immunity and other delayed complication like morphological changes in kidney and severe vascular complication (Defronzo et al., 1982; Jarvinen and Koivisto, 1984, 1986). Chronic treatment of sulfonylureas and biguanides are also associated with side effects (Rang et al., 1991) such as nausea and vomiting, agranulocytosis, aplastic and hemolytic anemia, dermatological reactions and lactic acidosis and generalized hypersensitivity reaction (Khan and Shechter, 1991). In India, use of herbal drugs based on Ayurveda is very commonly practiced from long past and it is less expensive. The herbal drugs we frequently considered are of less toxic with fewer side effects compared with synthetic drugs (Geetha et al., 1994; Rao et al., 2003). From such various reasons, at present traditional and complementary medicine has been an upsurge in its popularity for the treatment of different diseases. The National Centre for Complementary and Alternative Medicine which was established in 1998 by the United States Government where herbal medicine

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development is one of the important subjects of study (Yoon et al., 2004; Edwards et al., 2005). Though there are few reports on antidiabetic activity of Eugenia jambolana (E. jambolana) but till now no work has been performed to find out the hypoglycemic effect of E. jambolana and Musa paradisiaca (M. paradisiaca) in composite way. In Indian system of folk medicine, more than one plant in combined way are used for the correction of health disorders and this composite plant extract in the form of tonic or mixture exhibits a better results than single plant extract treatment (Wu et al., 1998; Borchers et al., 1999). Moreover, diabetic state is closely associated with oxidative injury (Kakkar et al., 1995) and there is no report about antioxidant potency of these plants in individual or in composite manner. On that background we used here the composite of two-plant material for the management of diabetes mellitus. From the use of different solvent extracts in our pilot work, it has been indicated that methanol extracts of these plants is comparatively more effective and exhibits a promising result. The specific dose and ratio of these plant used here is also most effective that we have standardized in our laboratory from trial and error by changing the ratio and dose of these two plant products. All the experiments were also repeated for three times. In the present study an approach has been made to reach the aim of finding out a cheapest and effective herbal antidiabetic drug especially for the poor community as a substitution of antidiabetic drugs and insulin therapy by forming its antihyperglycemic and antioxidantive mechanism in diabetic state.

Jamun (E. Jambolana) is used as traditional medicine for the management of diabetes (Kirtikar and Basu, 1991). It is found all over India. It belongs to the Myrtaceae family. Jambu bark cure hemorrhages, burning sensation, dysentery, diarrhea, diabetes, excessive thirst, dyspepsia, cough and asthma and is used in preparation of astringent decoction for gargles and washes (Joshi, 2000). Juice of ripe fruit, made into vinegar used as a stomachic, carminative and as diuretic (Joshi, 2000). Fruit is useful astringent in bilious diarrhea. Seeds are used for the treatment of diabetes (Joshi, 2000).

Kadali (M. paradisiaca) is a tree like herb and it is distributed throughout the India and Malaysia. This is belonging to the Musaceae family. Roots of Rambha are anthelmintic (Kirtikar and Basu, 1991). Flowers are astringent (Joshi, 2000). Banana fruit is mild laxative. It aids in combating diarrhea and dysentery and promotes healing of intestinal lesions in ulcerative colitis (Joshi, 2000). It is useful in celiac disease, constipation and peptic ulcer. Banana powder is effective in treatment of celiac disease (Joshi, 2000). Unripe fruit and cooked flower are useful in diabetes (Joshi, 2000).

MATERIALS AND METHODS

Chemicals: Streptozotocin was obtained from spectrochem Pvt. Ltd. chemical company (India). Insulin enzyme linked immunosorbent assay (ELISA) kit purchased from Boehringer Mannheim Diagnostic, Mannheim (Germany).

Plant material: The seeds of E. jambolana and root of M. paradisiaca were collected from Midnapore, district Paschim Midnapore, West Bengal in the month of June and the materials were identified by taxonomist of Botany Department, Vidyasagar University, Midnapore. The voucher specimen was deposited in the Department of Botany, Vidyasagar University and the voucher specimen numbers are HPCH No-6, 7.

Preparation of extract of E. jambolana: Fresh seeds of Eugenia jambolana were collected from rural areas in the month of June. These are dried in an incubator for 2 days at 40°C, crushed in an electric grinder and then powdered. Out of this powder, 50 g was suspended in 250 mL of methanol and kept at incubator at 37°C for 36 h. The slurry was stirred intermittently for 2 h and left overnight. The mixture was then filtered and filtrate was dried by low pressure and residue was collected. When required the residue was suspended in olive oil in a fixed dose and used for treatment.

Preparation of extract of M. paradisiaca: Fresh roots of M. paradisiaca were collected from local rural areas. These are cut into small pieces and dried in an incubator for 2-3 days at 40°C, crushed in an electric grinder and then powdered. Out of this powder 50 g was suspended in 250 mL of methanol and kept at incubator at 37°C for 36 h. The same procedure was followed like the E. jambolana.

Selection of animal and animal care: Forty matured Wistar strain male albino rats (Chakraborty Traders, Midnapore, India) of 3 month of age weighing about 150±10 g were taken for this experiment. Animals were acclimated for a period of 15 day in our laboratory condition prior to the experiment. Rats were housed in colony cage (4 rats per cage) at an ambient temperature of 25±2°C with 12 h light : 12 h dark cycle. Rats have free access to standard food and water ad libitum. The principles of laboratory animal care (NIH 1985) were followed through out the duration of experiment and instruction given by our institutional ethical committee was followed regarding injection and other treatment of experiment. Normoglycemic animals were selected for this.
experiment having the fasting blood glucose level of 70-80 mg dL$^{-1}$. In the month of November-December 2005, the study was conducted in the Research Laboratory of Reproductive Endocrinology and Family welfare, Vidyasagar University.

**Induction of diabetes mellitus:** Rats were fasted for 24 h before the induction of diabetes by streptozocin (STZ) injection. A freshly prepared solution of STZ at the dose of 4 mg 0.1 mL$^{-1}$ of citrate buffer 100 g body weight$^{-1}$ rat$^{-1}$ was injected intramuscularly. This single dose of STZ produce type I diabetes (having fasting blood sugar level more than 250 mg dL$^{-1}$) after 24 h of STZ injection and this diabetic state was maintained throughout the experimental schedule.

**Animal treatment:** Forty rats were divided into five groups as follows.

**Group I:** Control rats received single intramuscular injection of citrate buffer at the level of 0.1 mL 100 g body weight$^{-1}$ rat$^{-1}$.

**Group II:** Streptozocin diabetic rats of this group were made diabetic by single intramuscular injection of streptozocin induced at the dose of 4 mg 0.1 mL citrate buffer$^{-1}$ 100 g body weight$^{-1}$ rat$^{-1}$.

**Group III:** Streptozocin-induced diabetic rats of this group were forcefully fed by gavage method with methanolic extract of seed of *E. jambolana* at the dose of 80 mg 0.5 mL olive oil$^{-1}$ 100 g body weight$^{-1}$ day$^{-1}$ after 24 h of STZ injection for 14 days at fasting state.

**Group IV:** Streptozocin-induced diabetic rats were forcefully fed with methanolic extract of root of *M. paradisiaca* at the dose of 80 mg 0.5 mL olive oil$^{-1}$ 100 g body weight$^{-1}$ day$^{-1}$ for 14 days.

**Group V:** The diabetic rats of this group were forcefully fed with methanolic extract of seeds of *E. jambolana* and *M. paradisiaca* in composite manner at the dose of 80 mg (1:1) 0.5 mL olive oil$^{-1}$ 100 g body weight$^{-1}$ rat$^{-1}$ for 14 days. Extract co-administration in group III, IV and V was performed at early morning and at fasting state.

Animals of control (group I) and diabetic groups (group II) were subjected to forceful feeding of 0.5 mL of olive oil$^{-1}$ 100 g body weight$^{-1}$ day$^{-1}$ for 14 day at the time of extract co-administration to the animals of group III, IV and V to keep all the animal in same experimental condition.

From the starting day of extract supplementation to diabetic rats fasting blood glucose level of rats in all groups was measured by single touch glucometer at the interval of two days.

On 16th day of experiment, all the animals were sacrificed by decapitation after recording the final body weight, blood was collected from dorsal aorta and plasma was separated by centrifugation at 3000 g for 5 min for the assay of insulin followed by ELISA technique. Liver, kidney, skeletal muscle were dissected out and stored at -20°C for biochemical analysis of enzyme activity of glucose-6-phosphatase, glucose-6-phosphate dehydrogenase, hexokinase in liver and catalase, glutathione peroxidase activity in liver and skeletal muscle. Glycogen content and quantity of TBARS, conjugated dienes were quantified in liver and skeletal muscle.

**Testing of fasting blood glucose level:** At the time of grouping of the animals, Fasting Blood Glucose (FBG) level was measured. At 2-days interval FBG was further recorded from all the animals, of all groups. Blood was collected from the tip of tail vein and FBG level was measured by single touch glucometer.

**Biochemical assay of glucose-6-phosphatase activity in liver:** The liver glucose-6-phosphatase activity was measured according to standard protocol (Swanson, 1955). Tissue was homogenized in ice cold of 0.1 M phosphate buffer saline (pH-7.4) at the tissue concentration of 50 mg mL$^{-1}$. In a calibrated centrifuge tube, 0.1 mL of 0.1 M glucose 6-phosphate solution and 0.3 mL of 0.5 M 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 centrifuged at 3000 g for 10 min. The optical density was noted at 340 nm. The enzyme activity was expressed as mg of inorganic phosphate liberated per gm of tissue.

**Biochemical assay of glucose-6-phosphate dehydrogenase in liver:** The liver glucose-6-phosphate dehydrogenase activity was measured according to standard protocol (Langdon, 1956). Tissue was homogenized in ice-cold 0.1 M phosphate buffer saline (pH-7.4) at the tissue concentration of 50 mg mL$^{-1}$. In a spectrophotometric cuvette, 0.3 mL of 1 M Tris-chloride buffer (pH-7.5), 0.3 mL of 2.5×10$^{-2}$ M glucose-6-phosphate, 0.1 mL of 2×10$^{-2}$ 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 of 1 μM of NADP per minute.
Assay of hexokinase in liver: The enzyme activity was determined by the method of Chou and Wilson (1975) and was based on the reduction of NADPH coupled with hexokinase which was measured spectrophotometrically at 340 nm.

Biochemical assay of glycogen content: Glycogen levels in liver and skeletal muscle were measured according to the standard protocol (Sadasivam and Manickam, 1996). Liver and skeletal muscle tissues were homogenized separately in hot 80% ethanol at the tissue concentration of 100 mg mL⁻¹ and then centrifuged at 8000 g for 20 min. The residue was collected and allowed to dry over a water bath. To the residue, 5 mL of distilled water and 6 mL of 52% perchloric acid were added. The extraction was done at 0°C for 20 min. The collected material was centrifuged at 8000 g and supernatant was collected. From supernatant, 0.2 mL was transfer in graduated test tube and volume was made upto 1 mL by the addition of distilled water. Graded standards were prepared by using 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mL of working standard solution and volume of all these standards were made up to 1 mL by addition of distilled water. In all the test tubes 4 mL of anthrone reagent was added. The test tubes were kept in boiling water bath for 8 min. Then these were allowed to cool at room temperature and the intensity of green to dark green color of the solution was recorded at 630 nm. The amount of glycojen content was measured from standard curve prepared with standard glucose solution. The amount of glycogen in tissue sample was expressed in μg of glucose per mg of tissue.

Biochemical assay of Catalase (CAT): The activity of CAT of the above mentioned tissues were measured biochemically (Beers and Sizer, 1952). For the evaluation of CAT activity, liver and skeletal muscles from each animal were homogenized separately in 0.05 M Tris-HCl buffer solution (pH-7.0) at the tissue concentration of 50 mg mL⁻¹. These homogenized were centrifuged at 10,000 g at 4°C for 10 min. In spectrophotometric cuvette, 0.5 mL of 0.00035 M H₂O₂ and 2.5 mL of distilled water were mixed and reading of absorbance was noted at 240 nm. Liver and skeletal muscle supernatants were added at a volume of 40 μL and the subsequent six readings were noted at 30 sec interval.

Biochemical assay of glutathione peroxidase (Gpx): The glutathione peroxidase activity was measured according to the method (Paglia and Valentine, 1967). Liver and skeletal muscle tissues were homogenized in ice cold of 0.1 M phosphate buffer saline (pH -7.4) at the tissue concentration of 50 mg mL⁻¹ and centrifuged at 8000 g for 15 min. The reaction mixture contents 2.525 mL of 0.1 M L⁻¹ Tris-HCl buffer (pH-7.2), 75 μL of 0.04 M L⁻¹ GSH (reduced form), 100 μL of 0.1 M L nicotinamide adenine dinucleotide phosphate (NADPH) and 100 μL of glutathione reductase (0.24 unit). One hundred micro liters of supernatant was added to 2.8 mL of reactive mixture and incubated at 25°C for 5 min. The reaction was initiated by adding 100 μL of 0.75 mM L⁻¹ H₂O₂ and then its absorbance was measured at 340 nm for 5 min. The activity was expressed as nM NADPH oxidized mg tissue⁻¹min⁻¹ using a molar extinction co-efficient of 6.22×10³ (mM L⁻¹cm⁻¹)

Estimation of lipid peroxidation from the concentration of thioribarbituric acid reactive substance (TBARS) and conjugated dienes (CD): The liver and skeletal muscle were homogenized separately at the tissue concentration of 50 mg mL⁻¹ 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 min individually. 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 thioribarbituric acid in 75 mL of 0.25 N HCl with 15 g 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 taken in spectrophotometer cuvette and read at 535 nm (Okhawa et al., 1979).

Quantification of the CD was performed by a standard method (Slater, 1984). The lipids were extracted with chloroform-methanol (2:1) 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.

Plasma insulin level: Plasma insulin was measured by enzyme linked immunoorbant assay (ELISA) using the kit (Brugi et al., 1988) (Boehringer Mannheim Diagnostic, Mannheim, Germany). The intra assay variation was 4.96%. As the samples were run at a time, so there is no inter assay variation. The insulin level in plasma was expressed in μIU mL⁻¹.

Biochemical assay of Glutamate Oxaloacetate Transaminase (GOT) and Glutamate Pyruvate Transaminase (GPT): Liver and kidney tissues were homogenized separately in ice cold of 0.1 M-phosphate
buffer (pH 7.4) at the tissue concentration of 50 mg mL⁻¹. For the measurement of liver and kidney G6P and GPT kits were used supplied by Crest Biosystems, Gitanjali, Dr. Antonio Do Rego Bagh, Alto Sanctaracruz, Bambolin Complex (Goa, India). The activities of these enzymes were expressed as unit per gram of tissue (Henry et al., 1960).

**Statistical analysis:** Analysis of Variance (ANOVA) followed by multiple two-tail t-test was used for statistical analysis of collected data (Sokal and Rohle, 1997). Differences were considered significantly at p<0.05.

**RESULTS**

**Body weight:** Body weight was decreased significantly in streptozotocin-induced diabetic rats in respect to control. After the supplementation of methanolic extract of seed of *E. jambolana* and root of *M. paradisiaca* in separate manner this parameter was recovered significantly towards the control level. Body weight of the animal of composite group insignificantly differs from the control after the 14 days treatment of composite extract (Table 1).

**Fasting blood glucose level:** There was a significant elevation in fasting blood glucose level after single dose of injection of streptozotocin when compare to control. This parameter came to the control level gradually after supplementation of methanolic extract of seed of *E. jambolana* and root of *M. paradisiaca* in separate way but more significant recovery was noted after supplementation of composite extract (Table 2).

**Hexokinase activity:** Hepatic hexokinase activity was decreased significantly in diabetic group in respect to control. After the supplementation of these methanolic plant extract in separate way this enzyme activity was elevated towards the control level and in composite group this parameter insignificantly differ from control after the treatment of composite extract (Table 3).

**Glucose-6-phosphatase activity:** Hepatic glucose-6-phosphatase activity was increased significantly in diabetic group when compare to control. After treatment of these plant extracts in separate way, glucose-6-phosphatase activity was recovered significantly and this parameter resettled to the control level after the supplementation of composite extract (Fig. 1).

**Glucose-6-phosphate dehydrogenase activity:** Glucose-6-phosphate dehydrogenase activity in liver was decreased significantly in the streptozotocin-induced diabetic group in compare to control. After 14 days treatment of these two plant parts in separate way this parameter was recovered significantly in respect to diabetic group. After treatment of the composite extract this parameter was insignificantly differ from the control level (Fig. 1).

**Glycogen content:** Significant diminution was noted in the level of glycogen in liver and skeletal muscle in diabetic group in respect to control. After supplementation of earlier mentioned plant extract in separate manner to the diabetic animal, there was a significant recovery in this parameter when compare to diabetic group. Glycogen levels in above-mentioned tissues were restored to the control level after supplementation of composite extract of above plants (Fig. 2).

<table>
<thead>
<tr>
<th>Table 1: Effect of separate and composite methanolic extract of seed of <em>E. jambolana</em> and root of <em>M. paradisiaca</em> on body growth in streptozotocin-induced diabetic rats</th>
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<tr>
<td><strong>Body weight (g)</strong></td>
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<td><strong>Initial</strong></td>
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<td>Group I</td>
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<td>Group II</td>
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<td>Group IV</td>
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<td>Group V</td>
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Data are expressed as Mean±SEM, n = 8. ANOVA followed by multiple comparison two tail t-test. Values with different superscript (a,b,c,d) in each vertical column differ from each other significantly (p<0.05)

<table>
<thead>
<tr>
<th>Table 2: Effect of separate and composite methanolic extract of seed of <em>E. jambolana</em> and root of <em>M. paradisiaca</em> on fasting blood glucose level at two days interval throughout the experimental schedule in streptozotocin-induced diabetic rats</th>
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<tr>
<td><strong>Fasting blood glucose level (mg dl⁻¹)</strong></td>
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<td><strong>0 day</strong></td>
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<td>Group I</td>
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<td>Group V</td>
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Data are expressed as Mean±SEM, n = 8. ANOVA followed by multiple comparison two tail t-test. Values with different superscript (a,b,c,d) in each vertical column differ from each other significantly (p<0.05)
Fig. 1: Effect of separate and composite methanolic extract of seed of *E. jambolana* and root of *M. paradisiaca* on glucose-6-phosphatase and glucose-6-phosphate dehydrogenase activities in liver. Data are expressed as Mean±SEM, n = 8. ANOVA followed by multiple comparison two tail t-test. Bars with different superscripts (a, b, c) differ from each other significantly (p<0.05).

Table 3: Effect of separate and composite methanolic extract of seed of *E. jambolana* and root of *M. paradisiaca* on hexokinase activity in hepatic tissue in streptozotocin-induced diabetic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Hexokinase activity (µg mg of tissue⁻¹)</th>
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<tbody>
<tr>
<td>I</td>
<td>148.05±4.23^a</td>
</tr>
<tr>
<td>II</td>
<td>168.42±3.11^b</td>
</tr>
<tr>
<td>III</td>
<td>130.14±4.32</td>
</tr>
<tr>
<td>IV</td>
<td>132.15±3.21^b</td>
</tr>
<tr>
<td>V</td>
<td>146.98±3.61^a</td>
</tr>
</tbody>
</table>

Data are expressed as Mean±SEM, n = 8. ANOVA followed by multiple comparison two tail t-test. Values with different superscript (a, b, c) in each vertical column differ from each other significantly (p<0.05).

**Plasma insulin level:** Plasma insulin level was decreased significantly in diabetic group in respect to control. After 14 days treatment of these two-plant extract in separate way there was a significant elevation in plasma insulin level towards the control. This parameter resettled to the control level after supplementation of composite extract (Fig. 2).

**Catalase activity:** Activities of catalase in liver and skeletal muscle were decreased significantly in diabetic group in respect to control group. After the treatment of these plant extract in separate way the activities of catalase in above-mentioned tissues were significantly recovered towards the control when compare to diabetic group. After supplementation of composite extract to the diabetic animal, the above parameter in both liver and skeletal muscle was insignificantly differ from the control (Fig. 3).

**Glutathione peroxidase activity:** Glutathione peroxidase activities in liver and skeletal muscle were diminished significantly in streptozotocin-induced diabetic rat in respect to control. This parameter in above-mentioned tissues was recovered towards the control level significantly after supplementation of these plant extract in separate manner. After administration of composite plant extract the level of the parameter in both the tissues was insignificantly differ from the control (Fig. 3).

**Conjugated dienes level:** Levels of conjugated dienes in liver and skeletal muscle were significantly elevated in diabetic group in respect to control. The levels of above mentioned parameter in both liver and skeletal muscles were recovered significantly when compare to diabetic group after supplementation of individual extract. There was no significant difference when the above parameter in above-mentioned tissues was compared between control and composite extract supplemented groups (Fig. 4).
Fig. 2: Effect of separate and composite methanolic extract of seed of *E. jambolana* and root of *M. paradisiaca*, on glycogen content in liver and skeletal muscle and plasma insulin level. Data are expressed as Mean±SEM, n = 8. ANOVA followed by multiple comparison two tail t-test. Bars with different superscripts (a,b,c) differ from each other significantly (p<0.05).

Fig. 3: Effect of separate and composite methanolic extract of seed of *E. jambolana* and root of *M. paradisiaca*, on catalase and glutathione peroxidase activities in liver and skeletal muscle. Data are expressed as Mean±SEM, n = 8. ANOVA followed by multiple comparison two tail t-test. Bars with different superscripts (a,b,c) differ from each other significantly (p<0.05).

**Thiobarbituric acid reactive substance (TBARS) level:**
Thiobarbituric acid reactive substance (TBARS) levels in liver and skeletal muscle were increased significantly in diabetic group in respect to control. There was a significant recovery in above parameter both in liver and skeletal muscle after supplementation of individual extract of above plants to the diabetic animals in comparison to STZ induced diabetic group. After supplementation of composite extract to the diabetic animal, the level of this parameter in both liver and skeletal muscle was insignificantly differ from the control (Fig. 4).

**Glutamate Oxaloacetate Transaminase (GOT) and Glutamate Pyruvate Transaminase (GPT) level in liver and kidney:** Hepatic and renal GOT and GPT levels were increased significantly in diabetic group in respect
Fig. 4: Effect of separate and composite methanolic extract of seed of *E. jambolana* and root of *M. paradisiaca*, on TBARS and CD levels in liver and skeletal muscle. Data are expressed as Mean±SEM, n = 8. ANOVA followed by multiple comparison two tail t-test. Bars with different superscripts (a,b,c) differ from each other significantly (p<0.05).

Fig. 5: Effect of separate and composite methanolic extract of seed of *E. jambolana* and root of *M. paradisiaca*, on GOT and GPT in liver and kidney. Data are expressed as Mean±SEM, n = 8. ANOVA followed by multiple comparison two tail t-test. Bars with different superscripts (a,b,c) differ from each other significantly (p<0.05).

to control group. These two parameters in both liver and kidney were come towards the control level after the supplementation of methanolic extract of seeds of *E. jambolana* and root of *M. paradisiaca* in separate manner and more significant result was noted in case of composite extract treatment (Fig. 5).

**DISCUSSION**

The present study focus the comparative study of individual as well as composite extract of seed of *E. jambolana* and root of *M. paradisiaca* for the management of streptozotocin induced diabetes mellitus...
in albino rat. For such assessment we have studied fasting blood glucose levels along with glycogen content in liver and skeletal muscle and activities of some important carbohydrate metabolic enzymes. Moreover we have also assessed the oxidative stress condition in liver and skeletal muscles in different experimental groups as diabetes has a strong association with oxidative injury (Oberley, 1988).

The study selected STZ induced hyperglycemia as an experimental model because it is one of the best models to study the effect of the antidiabeticogenic agent (Curter et al., 1971) as well as in continuation to our previous work (Mallick et al., 2006).

The specific dose of the extract used and duration of the treatment adopted here were selected by trial and error where good promising results were noted without any metabolic toxicity induction.

Co-administration of the composite and separate extract of these plants parts resulted a significant correction of Fasting Blood Glucose (FBG) level in respect to STZ induced diabetic group and this recovery was more effective when treatment of composite extract was used which primarily focus the antidiabetic activity of these plant products. The actual mechanism of such antidiabeticogenic activity is not clear from this study but following possible dimensions may be enlighted.

Glucose-6-phosphate dehydrogenase is an important regulator of pentose phosphate path way (Kimura et al., 2005) for the maintenance of normal blood sugar level. This enzyme activity is diminished here in STZ - induced diabetic group as its activity is under insulin (Pari and Murugan, 2005). After supplementation of extract there was a significant recovery of the enzyme activity in liver. This may be another possible way for such anti diabeticogenic potency.

Liver is the main organ responsible for the maintenance of blood glucose homeostasis (Laursen et al., 2002) where hepatic glucose-6-phosphatase plays an important role (Hume et al., 2005). To focus the underlying biochemical mechanism of the action of antidiabeticogenic activities of these extracts, we measured the enzyme activities in liver in different groups. In diabetic group this level was elevated which is parallel to other workers (Baquer et al., 1998). After the supplementation of these extracts, there was a significant recovery of the enzyme activity that may be another possible way for antidiabeticogenic potency of these extract.

Hexokinase, an important enzyme for glucose utilization through glycolytic path way (Murphy and Anderson, 1974; Laakso et al., 1995). Activity of this enzyme in liver was elevated in STZ induced diabetic rat that is consistent with other report (Rathi et al., 2002). Extract of these plants were resulted a significant recovery of this enzyme, which results an elevation in glucose utilization that may be another mechanism to challenge the diabetes.

The above enzyme activities, which were recovered, showed a more potent correction after composite extract treatment and this was equal to control, which may be due to the direct effect of this extract and or by elevation of plasma insulin, which has been focused here by monitoring plasma level of insulin.

In STZ induced diabetes, glycogen level in liver and skeletal muscle were diminished here which is consistent to other reports (Groover et al., 2002) and it may be due to low level of insulin (Weber et al., 1966). After the extract supplementation in diabetic rat, there was a significant recovery in hepatic and skeletal muscular glycogen level towards the control level whereas degree of recovery was more significant after composite extract administration. This focuses one of the possible mechanisms of antidiabeticogenic action of this extract by modulating glycogen metabolism.

Diabetes is also associated with lipid peroxidation (Maxwell et al., 1997) as insulin secretion is closely associated with lipoxygenase derived peroxides (Walsh and Pek, 1988; Metz, 1984). Elevation in lipid per oxidation leading to islet cell damage in diabetes that diminishes insulin secretion, which has been supported here from plasma insulin level. Elevation in lipid per oxidation in diabetic state has been supported here from the elevation in TBARS and CD, important indicators of oxidative stress condition (Fraga et al., 1988; Dillard et al., 1978) both in liver and skeletal muscle, important metabolic tissues. The elevation in lipid per oxidation in above tissues in diabetic state has been supported here by the diminution of catalase and glutathione peroxidase, important antioxidant enzymes (Pillai and Gupta, 2005) in liver and skeletal muscle. Supplementation of these extract results significant elevation or restoration of the antioxidant enzymes followed by diminution of TBARS and CD levels to the control along with plasma level of insulin which proposed the another mechanism of antidiabetic effect of this extract. The correction of oxidative stress noted in diabetic condition has been noted more efficient after composite extract administration.

The plant extracts used here whether have any toxicity in general, we measured hepatic and renal GOT and GPT activities, important indicators of general toxicity assessment (Ghosh and Suryawanshi, 2001). There was significant diminution in these parameters after separate and composite extract treatment in respect to diabetic group, which suggest that these plant extract is out of any
toxicity induction and recovered the general toxicity that is noted in diabetic state.

Thus the present study shows that administration of composite extract of *E. jambolana* and *M. paradisiaca* is more effective than individual extract to challenge the diabetic state. The active ingredient(s) present here may recover the disorders in carbohydrate metabolism noted in diabetic state by protecting the oxidative stress induced tissue damage for such disorder and or by stimulating the carbohydrate regulatory enzyme activities in target organ or by stimulating and regenerating β cells in pancreas. The actual mechanism is not clear and further biochemical and pharmacological investigations are needed to isolate and identify the active ingredient(s) in the composite extract.

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REFERENCES


