



## Antioxidative Activities of Ethyl Acetate Fraction of Seed of *Eugenia jambolana* on Different Biological Tissues in Streptozotocin Induced Diabetic Rats

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### ABSTRACT

It is well known that most of the cellular constituents such as proteins, lipids and DNA are damaged by the reactive oxygen species. Now the study on phytomolecule having free radical scavenging activity is important field of research in our community. In this aspect, attention has been focused to evaluate the antioxidative activities of ethyl acetate fraction of seed of *Eugenia jambolana* (*E. jambolana*) in connection with glycemic control. The said fraction of *E. jambolana* was administered orally at the dose of 200 mg/kg of body weight/day for 35 days. To delineate the antioxidant activity we measured the specific activities of antioxidant enzymes (AOEs) and antioxidant molecule. We also measured the lipids, proteins and DNA in different biological tissues for the assessment of diabetes induced oxidative stress injury. Results focused that the damage of cellular lipids, proteins and DNA which caused by free radicals increased significantly ( $p < 0.05$ ) in diabetic group when compared with control group. Fraction treatment to the diabetic rats resulted significantly ( $p < 0.05$ ) improved the oxidative stress profile in all tissues when compared with diabetic animals. The study enlighten that the fraction is effective for the management of diabetes induced oxidative damage and there efficacy was compared with standard antidiabetic drug i.e. Glibenclamide.

**Keywords:** Antihyperglycemic, Antioxidative, *E. jambolana*, Oxidative stress, Streptozotocin.

### INTRODUCTION

Recently it is observed that most of the killer diseases or disorders such as malignancy, diabetes mellitus are closely related to reactive oxygen species (ROS) and lipid peroxidation.<sup>1</sup> When free radicals are generated, the levels of antioxidant vitamins and enzymes are reduced and finally the oxidative stress is produced. These free radicals interfere with biochemical processes and represent an essential part of aerobic life and metabolism.<sup>2</sup> So, different antioxidants having free radical scavenging activity are very essential for the management of free radical inducing diseases. Recent study on diabetes and its complications including oxidative stress is very important field of research in developing country.<sup>3</sup> In diabetes it is noted that oxygen free radicals generation is increased drastically of different pathways like protein glycation, polyol pathway and glycooxidation etc. and it is one of the causes for diabetes induced oxidative stress. Moreover cellular inherent antioxidant defense activity is depressed by hypoglycemia.<sup>4</sup> Oxidative stress induced cell damage are check through different biomarkers. Changes in these biomarkers are indicator of physiological process and can be used as an early warning against ensuing pathology. Lipid peroxidase can be measure for the estimation of damage lipids. The oxidative damage to protein can be estimated by estimating formation of proteins carbonyls and depletion in protein sulphahydrals.<sup>5</sup> Single strand breaks in DNA and 8-hydroxy-2'-deoxyguanosine (8OHdG) can be checked for DNA damage analysis.

Every cell has a primary antioxidant defense activity including antioxidant enzymes (AOEs), namely catalase

(CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX) and glutathione reductase (GR), and secondary defense constituting of antioxidant molecules such as reduced glutathione, uric acid, vitamin C,  $\alpha$ -tocopherol and so on. To examine the defense of cells against oxidative stress, one can measure both AOEs and antioxidant molecules. The susceptibility of a given organ or system to oxidative stress is a function of balance between pro-oxidant factors and those scavenging them.<sup>6,7</sup> In diabetes, as a result of persistent hyperglycemia, oxidative stress is induced because of both increased production of plasma free radicals concentration and sharp reduction of antioxidant defenses.<sup>8</sup> It further contributes to development of secondary complications. Different synthetic drug are used for the management of diabetes mellitus, which are not free of side effects.<sup>9</sup> To minimize the side effect phytomolecule having antidiabetic activity are important field of research in resent time. As these plant products are less toxic, less side effect and relatively low cost for that reason World health organization (WHO) also recommended the phytomedicines. In India, *E. jambolana* has been widely used as folk medicine from ancient time. Different parts of *E. jambolana* such as kernel, leaves and septum have a significant antihyperglycemic effect. The antihyperglycemic activity of ethyl acetate fraction of seed of *E. jambolana* has been reported previously by us<sup>10-12</sup>, as well as by others.<sup>13</sup> In the present study, we have investigated oxidative stress profile (OSP) in terms of both antioxidant defense and oxidative damage caused to cellular protein, lipid and DNA in different group of experimental animals.



## MATERIALS AND METHODS

### Bioactivity-guided solvent fractionation of hydro methanolic (40: 60) extract of seed of *E jambolana*

The seeds of *E. jambolana* were collected in rainy season (July–August) from the local market of Midnapore, West Bengal, India, and authenticated by the taxonomist in the Department of Botany and Forestry, Vidyasagar University, Midnapore, where the voucher specimen was preserved having Ref. No.- BMLSM-10/06. Crude extract preparation using hydro-methanol (40: 60) as solvent mixture followed by its fractionation using ethyl acetate was performed as per our previously reported method.<sup>11</sup>

### Selection of animal and animal care

Three months of age, weighing about 120 ± 10 gm of matured normoglycemic [having fasting blood glucose (FBG) level 80-90 mg/dl] wistar strain male albino rats were used for this experiment. Rats were housed at an ambient temperature of 25 ± 2°C with 12 h light: 12 h dark cycle. Rats were provided with protein rich standard feed and water *ad libitum*. The principle of laboratory animal care and instructions given by our Institutional Ethical Committee (IEC) which is in compliance with the guidelines of Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA), were followed throughout the experiment.

### Induction of diabetes mellitus

Diabetes was induced by a single intramuscular injection of streptozotocin (STZ) (Sigma Aldrich, USA) at the dose of 40 mg/kg of body weight. STZ was dissolved in 0.1 M citrate buffer (pH 4.5). Diabetes was confirmed 7 days after STZ injection by determining the FBG concentration. Only animals with FBG levels 300-350 mg/dl were considered for the experiment.<sup>14</sup>

### Design

Before the induction of diabetes the rats were divided into four groups of six animals each.

**Control group:** Rats of this group received single intramuscular injection of citrate buffer (1ml/kg body weight) and this group were subjected to oral administration of 1 ml distilled water/kg body weight/day for 35 days.

**Diabetic group:** Rats of this group were made diabetic by single intramuscular injection of STZ at a dose of 40 mg/kg body weight. Oral administration of distilled water was performed for 35 days to diabetic rat at a dose of 1 ml/kg body weight per day.

**Ethyl acetate fraction treated diabetic group:** Diabetic rats of this group were treated with ethyl acetate fraction of seed of *E. jambolana* at the dose of 200 mg/2 ml distilled water/kg of body weight/day for 35 days at fasting state.

**Glibenclamide treated diabetic group:** Diabetic rats of this group were treated with Glibenclamide at the dose of

20 mg/kg body weight/day for 35 days at fasting condition.

Single dose of ethyl acetate fraction was administered every day orally at morning (10.00 a.m.) using intragastric tube for 35 days. On 36<sup>th</sup> day of extract treatment (42<sup>nd</sup> day of STZ-injection) all animals were sacrificed by cervical decapitation. Before sacrificed, blood was collected from tail vein to check fasting blood glucose levels. After sacrificed blood was collected from ocular vein for estimation of 8OHdG in serum and urine was collected to measure 8OHdG. For measurement of oxidative stress profile parameters, pancreas, liver, spleen, skeletal muscle and kidney were dissected out from each animals.

### Measurement of fasting blood glucose level

After grouping the animals, the FBG level was measured. On every 7<sup>th</sup> day of treatment, FBG was further recorded from all the animals of all groups. Blood was collected from the tip of the tail vein and FBG level was measured by single touch glucometer (Bayer's Ascensia Entrust, Bayer, Germany).

### Isolation of different tissue

On the day of sacrifice, different organs of the animal were perfused with 0.1 M potassium phosphate buffer (pH 7.2), through an abdominal aorta to remove contaminating blood. Individual organs were also perfused with the same buffer. Pancreas, liver, spleen, skeletal muscle and kidney were removed, washed with 0.1 M potassium phosphate buffer, pH 7.2, weighed and homogenized in the same buffer to the final concentration of 100 mg of tissue per milliliter of buffer. These homogenates were centrifuged at 15000 rpm for 1 h at 4°C; supernatants were collected and stored at -20°C until use.

### Measurement of lipid peroxidation

Lipid peroxidation was measured in terms of nano moles of malondialdehydes (MDAs) formed per 100 mg tissue following the protocol of Heath and Packer.<sup>15</sup> One-millilitre tissue homogenate (100 mg tissue) was precipitated with trichloroacetic acid (TCA) to obtain a final concentration of TCA as 5%. To this 4 mL of thiobarbituric acid (TBA), reagent was added, and the samples were boiled at 95°C for 30 min and cooled immediately on ice. The pink colour representative of thiobarbituric acid-reactive substances was measured at 532 nm, and at 600 nm for non-specific absorbance.

### Measurement of protein carbonyls

Protein carbonyls from tissue homogenate were measured using the protocol of Dalle-Donne et al.<sup>16</sup> To the tissue homogenate, 0.75 mL of 20% TCA was added and centrifuged at 2500 rpm for 20 min. The supernatant was decanted and the pellet was resuspended either in 750 µl of 2 N HCl alone or in 750 µl of DNPH (2,4-dinitrophenylhydrazine) in 2 N HCl. This was thoroughly



vortex to resuspend the pellet. The tubes were incubated at room temperature for 1 h. To all the tubes, 750  $\mu$ L of 20% TCA was added and vortexed properly. It was then centrifuged at 2500 rpm for 20 min. The pellet was washed thrice with ethanol: ethyl acetate mixture (1 : 1). The supernatant was decanted, and the pellet was dried. To this pellet, 1 mL of 6 M guanidine hydrochloride was added, and the pellet was dissolved using a glass rod. The samples were centrifuged at 2500 rpm for 20 min, and the absorbance of supernatant was measured at 370 nm.

### Measurement of 8OHdG

8OHdG was estimated from nuclear and mitochondrial DNA from all the tissues using competitive enzyme-linked immunosorbent assay (ELISA) and monoclonal antibody against 8OHdG.<sup>17</sup>

### Nuclear DNA isolation

50–100 mg of tissue was homogenized in 500  $\mu$ L MSHE buffer (4.11 g Mannitol, 2.396 g sucrose, 52.06 mg 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) and 50 mg Bovine serum albumin (BSA) in 100 ml distilled water) using homogenizer. Nuclei were collected by centrifuging the homogenate at 1000 g for 15 min at 4<sup>o</sup>C. The nuclear pellet was washed thrice with MSHE buffer and once with T10E buffer. Nuclei were resuspended in lysis buffer and incubated at 37<sup>o</sup>C overnight. After one extraction with tris saturated phenol, supernatant was treated with 50  $\mu$ g/mL RNase at 37<sup>o</sup>C for 1 h. This was extracted twice with phenol–chloroform (1 : 1) and twice with chloroform–isoamyl alcohol (24 : 1). DNA was precipitated with two volumes of ice-cold ethanol after adjusting the salt concentration to 0.15 M NaCl at 20<sup>o</sup>C overnight. The DNA precipitates were air dried and dissolved in distilled water and quantitated by reading absorbance at 260 nm.<sup>18</sup>

### Mitochondrial DNA isolation

100 mg of tissue was homogenized in 500  $\mu$ L MSHE buffer using homogenizer. The homogenate was centrifuged at 3500 rpm for 15 min at 4<sup>o</sup>C.<sup>19</sup> The supernatant was taken in a fresh tube and again centrifuged at 7000 rpm for 15 min at 4<sup>o</sup>C. The mitochondrial pellet was washed thrice with MSHE buffer and once with 10 mM Tris–Cl/1 mM ethylene diamine tetra acetic acid (EDTA). For confirming the purity of mitochondrial preparation, specific activity of succinate dehydrogenase was checked. DNA was isolated from mitochondrial fraction by standard procedure.<sup>18</sup>

### Estimation of 8OHdG by competitive ELISA

For competitive ELISA, 100 ng of 8OHdG was added to each well. The plate was incubated with monoclonal antibody (1  $\mu$ g/mL) having either standard 8OHdG or DNA samples from different tissues (single stranded, 100 ng/well), urine (100  $\mu$ L of 1 : 100 dilution) and serum (100  $\mu$ L of 1:100 dilution) for 90 min at 37  $^{\circ}$ C. After five washings with phosphate buffer saline with twine 20 (PBS-T), the plate was incubated with 100  $\mu$ L (1 : 2000) of

goat anti-mouse IgG conjugated with biotin F(ab') fragment in phosphate buffer saline (PBS) at 37  $^{\circ}$ C for 30 min. After five washes of PBS-T, the plates were incubated with 100  $\mu$ L (1 : 10 000) avidin conjugated with peroxidase at 37  $^{\circ}$ C for 30 min. Finally, after three washings of PBS-T and two washings with phosphate citrate buffer (pH 5.0), 100  $\mu$ L of substrate solution containing 10% 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 0.06% H<sub>2</sub>O in phosphate citrate buffer was added to each well. Following 10-min 2 incubation, the absorbance was measured at 410 nm using Spectra max 250 ELISA reader (Certified Gene Tool, United States).<sup>17</sup> Standard graph of amount of 8OHdG versus absorbance at 410 nm was plotted, and from this, the values for samples were extrapolated.

### Measurement of antioxidant enzymes and antioxidant molecules

The tissue homogenates were used to estimate the levels of AOE, namely CAT and SOD and antioxidant molecules i.e. reduced glutathione.

#### Estimation of catalase

Catalase is a heme-containing enzyme, which catalyses dismutation of hydrogen peroxide into water and oxygen.<sup>20</sup> Decomposition of H<sub>2</sub>O<sub>2</sub> by CAT was measured spectrophotometrically at 240 nm. One unit of enzyme activity is defined as the amount of enzyme required to degrade 1  $\mu$ mole of H<sub>2</sub>O<sub>2</sub> in 1 min.

#### Estimation of superoxide dismutase

Photochemically reduced flavins generate oxygen free radicals upon reoxidation in air. The oxygen free radicals reduce nitroblue tetrazolium to blue-colored formazan.<sup>21</sup> Hence, amount of SOD is directly proportional to the reactive oxygen species (ROS) generated. One unit of enzyme is defined as the amount of enzyme required to reduce 1  $\mu$ mole of nitroblue tetrazolium to formazan in 1 min.

#### Estimation of reduced glutathione

Tissue homogenate was mixed with same volume of 2 M HClO<sub>4</sub>/4 mM EDTA.<sup>22</sup> The precipitated proteins were sedimented by centrifugation at 8000 g for 10 min at 4<sup>o</sup>C. The supernatant was neutralized by 2 M KOH/0.3 M 3-(N-morpholino) propanesulfonic acid (MOPS). The insoluble KClO<sub>4</sub> as removed by centrifugation at 8000 g for 10 min at 4<sup>o</sup>C. The cell extract/standard GSH solution in phosphate EDTA buffer (0.1 M phosphate, 20 mM EDTA), 910  $\mu$ L was mixed with 50  $\mu$ L of 4 mg/mL NADPH in 0.5% w/w NaHCO<sub>3</sub>, 20  $\mu$ L of 1.5 mg/mL 5,5'-dithiobis-2-nitrobenzoic acid in 0.5% NaHCO<sub>3</sub> and 20  $\mu$ L of GR (final concentration 6 U/mL). The increase in absorbance at 412 nm under these conditions is directly related to the sum of reduced and oxidized forms of glutathione in cell extract. For the determination of GSSG, the cell extract was treated with 4-vinyl pyridine to the final concentration of 0.1% v/v and then incubated for 1 h at room temperature. At this concentration, 4-vinyl pyridine



can react with all GSH without interfering GSSG, and again, glutathione content was determined as mentioned earlier. The amount of GSH could be determined by subtracting the GSSG content from total glutathione. Total glutathione is expressed as micro molar per gram wet weight and is an average of three different experiments. The intraset coefficient of variation is less than 5%, and interest coefficient of variation for all the groups is less than 20%.

### Statistical analysis

Analysis of variance (ANOVA) followed by a multiple comparison two-tail *t*-test was used for statistical analysis of collected data. Differences were considered significant

at  $P < 0.05$ . All the values have been indicated by mean  $\pm$  SEM.<sup>23,24</sup>

## RESULTS

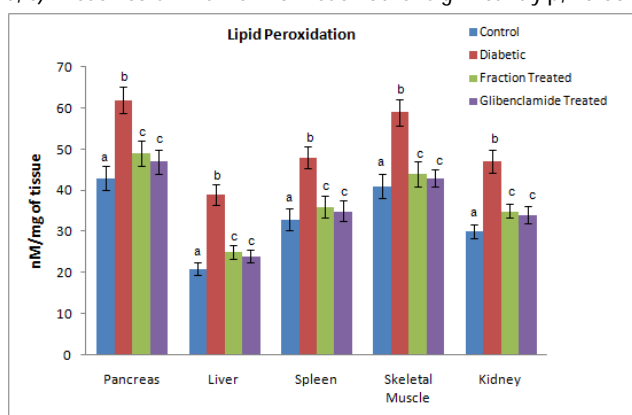
### Fasting blood glucose level

A significant elevation was noted on FBG levels in STZ-induced diabetic rats in compare to the control group. Administration of ethyl acetate fraction for 35 days resulted a significant ( $p > 0.05$ ) decrease in FBG level in the diabetic animals towards control levels. There was no significant difference in the levels of FBG in between Glibenclamide treated and ethyl acetate fraction treated animals (Table 1).

**Table 1:** Fasting blood glucose levels in control and different experimental group of animals

Groups	Fasting blood glucose level (mg / dl)						
	1 <sup>st</sup> day	8 <sup>th</sup> day	15 <sup>th</sup> day	22 <sup>nd</sup> day	29 <sup>th</sup> day	35 <sup>th</sup> day	42 <sup>nd</sup> day
Control	85.83 $\pm$ 4.8	86.83 $\pm$ 3.3	85.83 $\pm$ 4.7	82.5 $\pm$ 4.1	86.32 $\pm$ 4.7	86.32 $\pm$ 4.7	86.32 $\pm$ 4.7
Diabetic	86.66 $\pm$ 4.7	346.6 $\pm$ 8.2 <sup>a</sup>	350.33 $\pm$ 4.1 <sup>a</sup>	344.83 $\pm$ 2.2 <sup>a</sup>	342.33 $\pm$ 2.6 <sup>a</sup>	342.33 $\pm$ 2.6 <sup>a</sup>	342.33 $\pm$ 2.6 <sup>a</sup>
Fraction treated	85.16 $\pm$ 6.5	335.33 $\pm$ 6.4 <sup>b</sup>	283 $\pm$ 2.6 <sup>b</sup>	234.81 $\pm$ 2.9 <sup>b</sup>	150 $\pm$ 3.8 <sup>b</sup>	119 $\pm$ 3.8 <sup>b</sup>	90 $\pm$ 3.8 <sup>b</sup>
Glibenclamide treated	87.66 $\pm$ 4.6	324.66 $\pm$ 5.0 <sup>c</sup>	277.5 $\pm$ 3.6 <sup>c</sup>	228.33 $\pm$ 3.2 <sup>c</sup>	148.8 $\pm$ 4.1 <sup>b</sup>	118.8 $\pm$ 4.1 <sup>b</sup>	88.8 $\pm$ 4.1 <sup>b</sup>

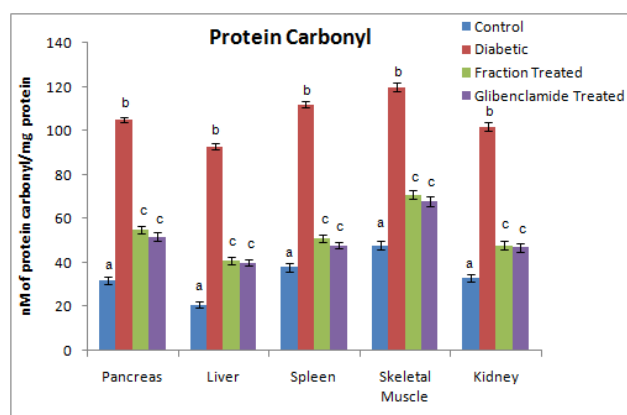
Data expressed as Mean  $\pm$  SEM, (n = 6), ANOVA followed by multiple comparisons two tail '*t*' test. Values with different superscripts (a, b, c) in each column differ from each other significantly  $p < 0.05$ .



**Figure 1:** Oxidative stress induced damage to cellular lipids in pancreas, liver, spleen, skeletal muscle and kidney homogenate from different experimental animals. Data expressed as Mean  $\pm$  SEM, (n = 6), ANOVA followed by multiple comparisons two tail '*t*' test. Values with different superscripts (a, b, c) in each column differ from each other significantly  $p < 0.05$ .

### Levels of lipid peroxidation

Levels of thiobarbituric acid reactive substances was significantly ( $p > 0.05$ ) higher in diabetic group in comparison to control group. Fraction treated and Glibenclamide treated group showed significant ( $p > 0.05$ ) recovery in compared to untreated diabetic group. But when comparison made between fraction treated and Glibenclamide treated group there was no significant ( $p > 0.05$ ) difference was noted. The amount of MDA formed was lowest in liver and highest in pancreas amongst all tissues in all the conditions (Figure 1).



**Figure 2:** Amounts of protein carbonyl in pancreas, liver, spleen, skeletal muscle and kidney homogenate from different experimental animals. Data expressed as Mean  $\pm$  SEM, (n = 6), ANOVA followed by multiple comparisons two tail '*t*' test. Values with different superscripts (a, b, c) in each column differ from each other significantly  $p < 0.05$ .

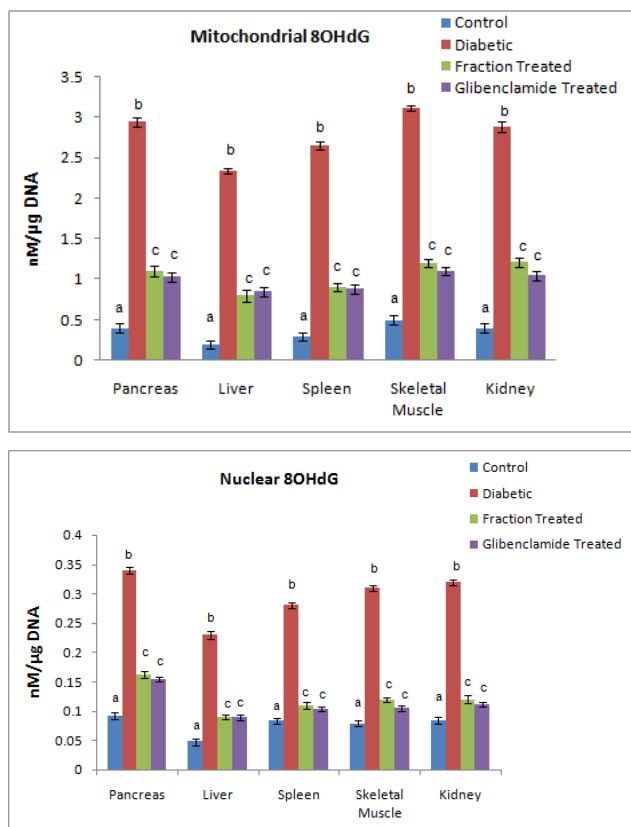
### Measurement of protein carbonyls

The amount of protein carbonyls formed was significantly higher ( $p > 0.05$ ) in diabetic animals in compared to control group. The amount of protein carbonyls formed was lowest in liver and highest in skeletal muscle. Animals of fraction treated group showed significant decrease ( $p > 0.05$ ) in protein carbonyl formation in compare to untreated diabetic group. But no significant difference was noted between fraction treated and Glibenclamide treated group (Figure 2).

### Levels of 8OHdG

We measured mitochondrial and nuclear 8OHdG, as a standard marker for oxidative DNA damage. DNA was isolated from nuclei and mitochondria of pancreas, liver, spleen, skeletal muscle and kidney tissues from animals. Amount of 8OHdG increased significantly ( $p > 0.05$ ) in mitochondrial DNA of all tissues of diabetic animals. The highest increase was observed in case of skeletal muscle followed by pancreas and kidney. After fraction treatment the amount of 8OHdG decreased significantly ( $p > 0.05$ ) in all the tissues when compared with untreated diabetic animals (Figure 3).

The amount of 8OHdG in the nuclear DNA of all the tissues was higher ( $p > 0.05$ ) under diabetic condition in respect to control group. Highest amount of 8OHdG was found in pancreas and lowest in liver. Fraction treatment reduced the amount of 8OHdG in DNA from all the tissues significantly ( $p > 0.05$ ); however, it did not reach the control levels (Figure 3). The amount of free 8OHdG excised from damaged DNA showed significant increase ( $p > 0.05$ ) in urine and serum of diabetic animals when compared to control group. Fraction treatment showed a significant decrease ( $p > 0.05$ ) in the amount of 8OHdG in urine and serum, respectively (Figure 4). But in all cases there was no significant difference was noted between fraction treated and Glibenclamide treated group.

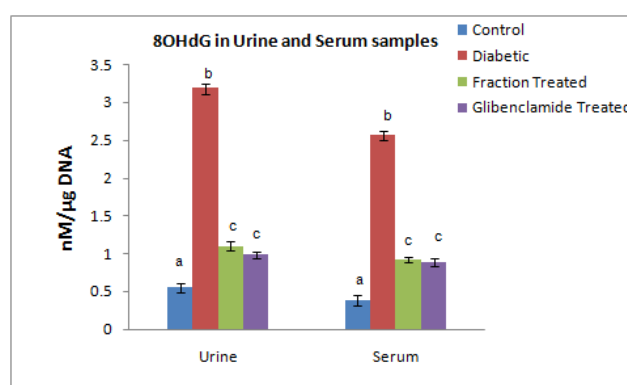


**Figure 3:** Amounts of 8OHdG in mitochondrial and nuclear DNA in pancreas, liver, spleen, skeletal muscle and kidney tissues from different experimental animals. Data expressed as Mean  $\pm$  SEM, (n = 6), ANOVA followed by multiple comparisons two tail 't' test. Values with

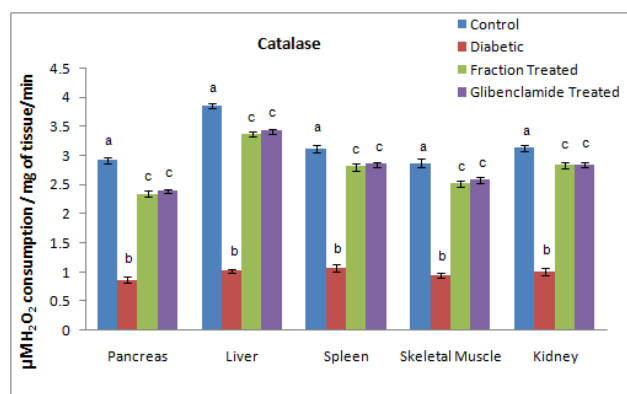
different superscripts (a, b, c) in each column differ from each other significantly  $p, < 0.05$ .

### Activities of antioxidant enzymes

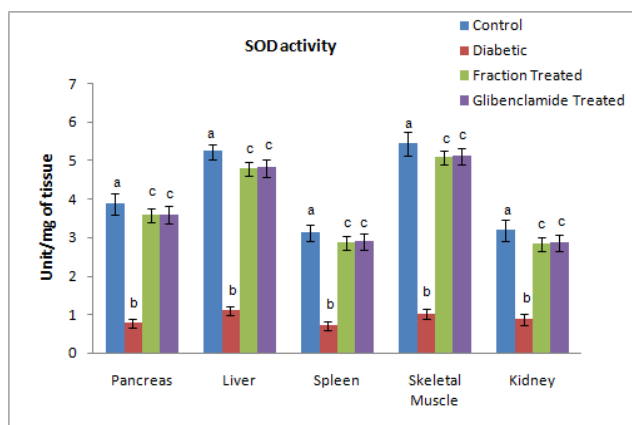
The activities of AOE, namely SOD and CAT was measured from tissue homogenates supernatant of pancreas, liver, spleen, skeletal muscle and kidney in various groups. It is clear that CAT (Figure 5) and SOD (Figure 6) decreased significantly ( $p > 0.05$ ) in diabetic group in compared with those of the control group. This decrease was highest in case of pancreas for CAT and in case of spleen for SOD. In fraction treated group of animals, activities of all the enzymes were significantly ( $p > 0.05$ ) recovered in compared to diabetic group which almost similar to those of the control group. There was no significant ( $p > 0.05$ ) difference was noted between fraction treated and Glibenclamide treated group.



**Figure 4:** Amounts of 8OHdG in urine and serum samples from different experimental animals. Data expressed as Mean  $\pm$  SEM, (n = 6), ANOVA followed by multiple comparisons two tail 't' test. Values with different superscripts (a, b, c) in each column differ from each other significantly  $p, < 0.05$ .



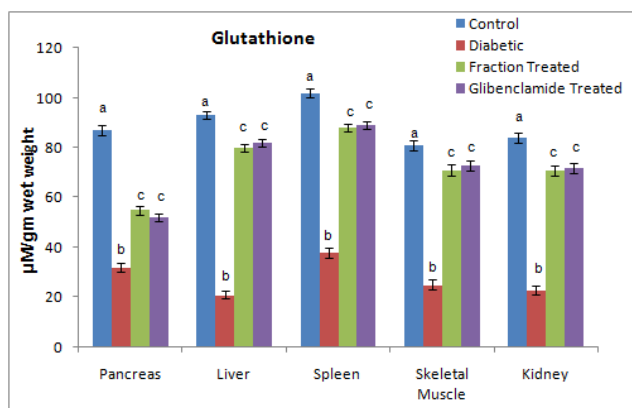
**Figure 5:** Levels of catalase enzyme in pancreas, liver, spleen, skeletal muscle and kidney tissues in different experimental animals. Data expressed as Mean  $\pm$  SEM, (n = 6), ANOVA followed by multiple comparisons two tail 't' test. Values with different superscripts (a, b, c) in each column differ from each other significantly  $p, < 0.05$ .



**Figure 6:** Levels of SOD enzyme in pancreas, liver, spleen, skeletal muscle and kidney tissues in different experimental group of animals. Data expressed as Mean  $\pm$  SEM, (n = 6), ANOVA followed by multiple comparisons two tail 't' test. Values with different superscripts (a, b, c) in each column differ from each other significantly p, <0.05.

### Measurement of antioxidant molecules

The amount of reduced glutathione was found to be decreased significantly ( $p > 0.05$ ) in all the tissues of diabetic animals. In fraction treated group of rats, the amount of GSH was significantly recovered in compared to diabetic group (Figure 7)



**Figure 7:** Activities of reduced glutathione in pancreas, liver, spleen, skeletal muscle and kidney tissues in different group of experimental animals. Data expressed as Mean  $\pm$  SEM, (n = 6), ANOVA followed by multiple comparisons two tail 't' test. Values with different superscripts (a, b, c) in each column differ from each other significantly p, <0.05.

### DISCUSSION

Recently management of diabetes without side effects is still a challenge in medical system. To minimize these side effects is an increasing demand to search out the natural plant products with antidiabetic activity. These plant products or phytomolecule reduced the blood sugar level through different mechanism. Some of them have insulin like substances and some may inhibit insulin's activity.<sup>25</sup> Few plants are involved to produce more insulin by

stimulating the  $\beta$  cell and few plants are involved to increase the  $\beta$  cell by regenerating the pancreatic cell.<sup>26,27</sup>

The said fraction decreased the glucose level in STZ induced rats. Such an effect may be implicit that the fraction may result in stimulations of the remaining  $\beta$ -cells of the pancreas in diabetic rats for insulin secretion or it may help in the regeneration of pancreatic  $\beta$ -cells which is in agreement with other reports<sup>14</sup>, as well as our previous reports on this aspect using other plant parts.<sup>10</sup>

Earlier studies have considered one or two parameters indicative of oxidative stress in diabetic animals.<sup>28, 29</sup> In this study, we have looked at the entire OSP including oxidative damage caused to the major biological molecules along with alternations in the endogenous antioxidant defense in different tissues of normal, diabetic, fraction treated and Glibenclamide treated diabetic rat. Oxidative damage was estimated by measuring lipid peroxidation, protein carbonyls and the amount of 8OHdG in DNA while antioxidant defense was measured by estimating AOE and antioxidant molecules.

It was observed that the damage caused by free radicals to cellular lipids, proteins and DNA increased substantially in diabetic animals compared with that in the controls. In case of diabetic animals, damage to all biological molecules increased further significantly ( $p > 0.05$ ). The ratio of MDA and protein carbonyls formed in diabetic and control animals was highest in the case of pancreas, which further increased in untreated diabetic pancreas indicating maximum damage to the pancreatic tissue. When we compared between nuclear and mitochondrial DNA, more damage was seen in mitochondrial DNA consistent with earlier report.<sup>30</sup> This higher oxidative damage to mitochondrial DNA is due to the fact that mitochondrial respiratory chain is the source of a continuous flux of oxygen radicals<sup>31</sup> and has easy accessibility to ROS compared with nuclear DNA and lower efficiency to repair the DNA damage.<sup>32,33</sup> Comparing the damage to lipids, proteins and DNA in different tissues under control, diabetic, fraction treated and Glibenclamide treated animals, we find that the liver showed lowest damage owing to its strong antioxidant defense. In case of proteins and lipids, skeletal muscle showed higher damage, whereas DNA damage was significantly high in pancreas under all conditions. This higher oxidative damage to mitochondrial DNA is due to the fact that mitochondrial respiratory chain.

Superoxide dismutase and catalase are considered as primary enzymes since they are involved in the direct elimination of oxygen species,<sup>34</sup> Superoxide dismutase is an important defense enzyme, which catalyses the dismutation of superoxide radicals<sup>35</sup>, and catalase is a hemoprotein, which catalyses the reduction of hydrogen peroxides and protects tissues from highly reactive hydroxyl radicals. The reduced activity of superoxide dismutase and catalase in the liver and kidney observed in diabetes may pose deleterious effects as a result of the

accumulation of superoxide anion radicals and hydrogen peroxide.

Treatment of said fraction showed decreased lipid peroxidation, which is associated with increased activity of SOD and CAT which indicate that the extract can reduce reactive oxygen free radicals and improve the activities of the hepatic antioxidant enzymes.<sup>36, 37</sup>

Antioxidative activity of the said fraction has been strengthened here from the quantification of TBARS in hepatic tissues as there is an inverse relationship between the activities of antioxidant enzymes and quantity of free radical, which is in consistent with previous reports.<sup>38</sup>

## CONCLUSION

Present study convey the novel information on the antioxidant activity of *E. jambolana* in STZ induced diabetic rats and provides more evidence that the protective effects are, possibly, due to a decline in oxidants by the hepatic tissues. Further the oral administration of *E. jambolana* STZ induced diabetic rats exhibited significant ameliorative potential probably by attenuating the hyperglycemia-mediated oxidative stress. Further detailed studies are in progress to elucidate the exact mechanism by which *E. jambolana* elicits its modulatory effects.

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