Evaluation of in vivo Antioxidant Potential of the Aerial Parts of Aerva lanata Linn Juss in Streptozotocin Induced Oxidative Stress Rats

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Abstract

To evaluate the in vivo antioxidant activities of methanol (MEAL) and aqueous extracts (AEAL) of aerial parts of Aerva lanata Linn Juss in streptozotocin induced oxidative stress rats which are used in the folklore system for the treatment of diabetes mellitus in India. Rats were divided into seven groups of six rats (n=6) each. Group 1 served as vehicle control using normal saline (0.9% w/v NaCl), Group 2 served as diabetic control, Group 3 served as standard treated with 0.5 mg/kg of glibenclamide, Group 4 and 5 was treated with MEAL 200 and 400 mg/ kg, p.o., Group 6 and 7 was treated with AEAL 200 and 400 mg/ kg, p.o. Phenolic content, antioxidant enzymes, liver glycogen and lipid peroxidation were evaluated and compared with diabetic control. MEAL (400 mg/ kg) showed significant increase in the protein, catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), reduced glutathione (GSH), hexokinase and liver glycogen levels (p < 0.01) than AEAL when compared with diabetic control. The MEAL at the dose of 400 mg/ kg showed significant decrease in thiobarbituric acid reactive substance (TBARS) levels, Glucose 6-phosphatase (G6-pase), Fructose 1,6 biphosphatase (FD-pase). Thus, these results scientifically confirm that MEAL at the higher dose may effectively normalize the various disorders caused by free radicals due to the presence of secondary metabolites that exert antioxidant activity.

Keywords Aerva lanata; Oxidative stress; Streptozotocin; Diabetes; Lipid peroxidation

Background

Natural products play a significant role in maintaining human health by improving the quality of human life and have served valuable components of medicines, cosmetics, dyes and beverages to the human. Demand for medicinal plant is increasing in both developing and developed countries due to growing recognition of natural products, since it is having fewer side effects as compared to that of synthetic compounds. It is readily available for low price and sometime the only source of health care available to the poor. The World Health Organization (WHO) estimates that 80% of the populations living in the developing countries rely exclusively on traditional medicine for their primary health care needs.

Diabetes mellitus is a group of metabolic disorders characterised by high blood glucose level due to decrease in the level of insulin. Long term complications of this illness contribute to increase mortality and morbidity. Worldwide about 371 million people are affected and by 2035 the number is predicted to go up to almost 600 million (Zimmet et al., 2014). Asia has emerged as a “diabetic hub”. In India 61.3 million people live with diabetes. More than 90% people live with Type 2 DM (Chen., 2012). Diabetes mellitus is the sixth leading cause of death globally (Nash et al., 2001). There is increasing evidence that complications of diabetes are due to oxidative stress induced by the generation of oxygen free radicals and a sharp reduction of antioxidant defences (Oberley., 1988). Streptozotocin induced diabetes mellitus is associated with oxidative damage due to the generation of reactive oxygen species (Szukudelski., 2001). The chronic hyperglycemia was found to increase the production of free radicals that is associated with long term damage, dysfunction and failure of various organs like eyes, kidneys, nerves, heart and blood vessels (Baynes et al., 1977).
Several hypothesis have been reported to explain the genesis of free radicals in diabetes and these include oxidation of glucose, non enzymatic and progressive glycation of proteins with consequently increased formation of glucose derived advanced glycation end products (AGEs) (Vlassara et al, 2001). Common advantages of herbal medicines are effectiveness, safety, affordability and accepted by large group of people (Valiathan., 1998). Furthermore, World Health Organization (WHO) has also recommended the medicinal plants for the treatment of diabetes (WHO., 1980). Patients are therefore using herbal medicines which impart therapeutic effect in complicated disorders like diabetes and its complication with fewer side effects (Pandita et al., 2010). Many plant extracts have been shown to have significant antioxidant activity and useful in treatment of several diseases including diabetes (Scartezzini et al., 2002). Evidences indicate that free radicals, membrane lipid peroxidation and protein oxidation are significantly increased in diabetic patients and in experimental diabetic animals (Vandam et al., 1997).

_Aerva lanata_ Linn Juss belongs to the family Amaranthaceae is an important source of chemicals of immense pharmaceutical importance. The plant is distributed throughout tropical India as a common weed in fields and wasteland and is also found to be grown in Arabia, Tropical Africa, Sri Lanka, Philippines and Java (Krishnamurthi., 2003). The literature survey reveals that few work has been carried out on _Aerva lanata_, however some antidiabetic activity are still without scientific backing. The present study was undertaken to assess the MEAL and AEAL on total protein, antioxidant enzymes, liver glycogen and lipid peroxidation in streptozotocin induced diabetic rats.

## 1 Results

### 1.1 Acute toxicity study

The various observations showed the normal behavior of the treated rats. Toxic effects were not observed at a higher dose of 4 g/ kg body weight. Hence, there was no lethal effect in any of the groups.

### 1.2 Effect of MEAL and AEAL on lipid peroxidation (TBARS)

The level of MDA gives the picture of thiobarbituric acid reactive substances. The level of MDA was significantly higher with diabetic control animals. The level of MDA was significantly ($p < 0.01$) lowered on treatment with MEAL and AEAL (Table 1).

<table>
<thead>
<tr>
<th>Treatment (mcg/ mg. prtn/ml)</th>
<th>MDA (mg/ dl)</th>
<th>Liver Glycogen (mg/ dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>10.63 ± 0.21</td>
<td>689.34 ± 24.93</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>12.94 ± 0.37</td>
<td>527.53 ± 7.08</td>
</tr>
<tr>
<td>Standard</td>
<td>10.01 ± 0.28**</td>
<td>681.72 ± 33.85**</td>
</tr>
<tr>
<td>MEAL (200 mg/ kg)</td>
<td>9.25 ± 0.06**</td>
<td>674.15 ± 32.66**</td>
</tr>
<tr>
<td>MEAL (400 mg/ kg)</td>
<td>9.92 ± 0.12**</td>
<td>679.64 ± 22.22**</td>
</tr>
<tr>
<td>AEAL (200 mg/ kg)</td>
<td>8.87 ± 0.18**</td>
<td>661.29 ± 33.66*</td>
</tr>
<tr>
<td>AEAL (400 mg/ kg)</td>
<td>9.07 ± 0.21**</td>
<td>665.18 ± 32.80**</td>
</tr>
</tbody>
</table>

Note: Values are expressed as mean ± SEM ($n = 6$) in each group. Values were found out by using one way anova followed by Dunnet's test. ** Values were significantly different from hyperglycemic control at $p < 0.01$. * Values were significantly different from hyperglycemic control at $p < 0.05$

### 1.3 Effect of MEAL and AEAL on total protein

There was significant decrease in total protein in diabetic rats. Treatment with MEAL and AEAL significantly increase ($p < 0.01$) the total protein (Table 2).
Table 2 Effect of MEAL on protein, catalase, SOD and GPx

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Protein (mg/ ml)</th>
<th>CAT (mcg/ mg. prtn/ ml)</th>
<th>SOD (unit/ mg prtn)</th>
<th>GPx (mcg/ mg. prtn/ ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>9.38 ± 0.51</td>
<td>12.44 ± 0.14</td>
<td>11.97 ± 0.40</td>
<td>11.52 ± 0.48</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>6.63 ± 0.18</td>
<td>5.56 ± 0.19</td>
<td>6.52 ± 0.16</td>
<td>7.89 ± 0.19</td>
</tr>
<tr>
<td>Standard</td>
<td>8.81 ± 0.28</td>
<td>10.6 ± 0.22 **</td>
<td>10.24 ± 0.50**</td>
<td>11.61 ± 0.89**</td>
</tr>
<tr>
<td>MEAL (200 mg/ kg)</td>
<td>8.17 ± 0.14**</td>
<td>8.98 ± 0.14**</td>
<td>9.78 ± 0.26**</td>
<td>10.73 ± 0.36**</td>
</tr>
<tr>
<td>MEAL (400 mg/ kg)</td>
<td>8.44 ± 0.17**</td>
<td>10.52 ± 0.22 **</td>
<td>10.40 ± 0.22**</td>
<td>10.99 ± 0.31**</td>
</tr>
<tr>
<td>AEAL (200 mg/ kg)</td>
<td>7.92 ± 0.08**</td>
<td>8.55 ± 0.23 **</td>
<td>8.31 ± 0.40**</td>
<td>10.34 ± 0.42**</td>
</tr>
<tr>
<td>AEAL (400 mg/ kg)</td>
<td>8.04 ± 0.02**</td>
<td>9.98 ± 0.16 **</td>
<td>8.59 ± 0.33**</td>
<td>10.38 ± 0.22**</td>
</tr>
</tbody>
</table>

Note: Values are expressed as mean ± SEM (n=6) in each group. Values were found out by using one way anova followed by Dunnet's test. ** Values were significantly different from hyperglycemic control at p < 0.01. * Values were significantly different from hyperglycemic control at p < 0.05

1.4 Effect of MEAL and AEAL on antioxidant enzymes

The level of antioxidant enzymes viz. CAT, SOD, GPX, GSH and hexokinase has significantly decreased in the diabetic control animals. The treatment with MEAL and AEAL has significantly (p < 0.01) increased the levels of CAT, SOD, GPX, GSH and hexokinase.

1.5 Effect of MEAL and AEAL on hepatic glucose – 6 – phosphatase and fructose 1, 6 – bisphosphatase

The level of hepatic glucose-6-phosphatase and fructose 1, 6 – bisphosphatase was significantly increased in the diabetic control animals. The MEAL and AEAL treatment has significantly (p < 0.01) decreased the level of glucose-6-phosphatase and fructose 1, 6 – bisphosphatase in the liver (Table 3).

Table 3 Effect of MEAL on GSH, G 6-Pase, FD Pase, Hexokinase

<table>
<thead>
<tr>
<th>Treatment</th>
<th>GSH (mg/ dl)</th>
<th>G 6-Pase (mcg/ prtn/ ml)</th>
<th>FD Pase (mcg/ mg. prtn/ ml)</th>
<th>Hexokinase (mcg/ mg. prtn/ ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>61.48 ± 0.35</td>
<td>568.77 ± 1.58</td>
<td>667.75 ± 5.73</td>
<td>814.96 ± 19.29</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>50.11 ± 0.58</td>
<td>645.34 ± 9.76</td>
<td>778.60 ± 6.85</td>
<td>673.82 ± 14.48</td>
</tr>
<tr>
<td>Standard</td>
<td>59.33 ± 0.31**</td>
<td>592.08 ± 2.48**</td>
<td>737.45 ± 4.38**</td>
<td>784.40 ± 21.97**</td>
</tr>
<tr>
<td>MEAL (200 mg/ kg)</td>
<td>58.56 ± 0.32**</td>
<td>585.55 ± 1.11**</td>
<td>746.51 ± 4.4**</td>
<td>768.78 ± 19.56**</td>
</tr>
<tr>
<td>MEAL (400 mg/ kg)</td>
<td>58.79 ± 0.63**</td>
<td>584.66 ± 1.5 **</td>
<td>745.47 ± 4.4**</td>
<td>780.90 ± 21.35**</td>
</tr>
<tr>
<td>AEAL (200 mg/ kg)</td>
<td>54.64 ± 0.84**</td>
<td>607.56 ± 4.65**</td>
<td>754.03 ± 4.3**</td>
<td>759.94 ± 19.94**</td>
</tr>
<tr>
<td>AEAL (400 mg/ kg)</td>
<td>57.31 ± 0.55**</td>
<td>590.79 ± 1.79**</td>
<td>753.19 ± 3.3**</td>
<td>763.79 ± 17.22**</td>
</tr>
</tbody>
</table>

Note: Values are expressed as mean ± SEM (n = 6) in each group. Values were found out by using one way anova followed by Dunnet's test. ** Values were significantly different from hyperglycemic control at p < 0.01. * Values were significantly different from hyperglycemic control at p < 0.05

1.6 Effect of MEAL and AEAL on glycogen content in liver

The mobilization of glucose into liver and skeletal muscle was increased with experimentally induced diabetic rats. The level of hexokinase and glycogen content of liver was significantly decreased in the diabetic control animals. Treatment with MEAL and AEAL has significantly (p < 0.01) increased the level of liver glycogen content (Table 3).

2 Discussion

In light of the results, treatment with different doses of the extract was well tolerated by all the animals, as there were no toxic effects observed by direct visual observation of the animals throughout the experiment. This might suggest the non toxic effect of the extract.
It was observed that the levels of antioxidant enzymes like SOD, CAT, GPx, and GSH were decreased in kidney of diabetic rats. In diabetic rats treated with MEAL and AEAL, a significant increase in activity of these enzymes was observed, that is the levels were brought back to normal, indicate oxidative stress elicited by STZ had been nullified due to the effect of MEAL and AEAL (Table 1, Table 2). Streptozotocin induced diabetic rats showed decrease in the plasma protein content due to progressive protenuria followed by a gradual decline in renal function (Latha et al., 2011). The increased level of plasma protein in MEAL and AEAL treated diabetic rats suggested its remedial role on renal function. Free radicals are formed disproportionately in diabetes mellitus by glucose degradation, non-enzymatic glycation of proteins and the subsequent oxidative degradation. There is evidence that glycation itself induces the generation of oxygen-derived free radicals in diabetic condition (Senthilkumar et al., 2006). The generation of free radicals may lead to lipid peroxidation in diabetes mellitus (Mahboob et al., 2005).

 Associated with the changes in lipid peroxidation, diabetic animals showed decreased activity of the key antioxidant enzymes viz. SOD, CAT, reduced GSH and Glutathione peroxidase which play an important role in scavenging the toxic intermediates of incomplete oxidation. A decrease in the activity of these enzymes can lead to an excess availability of superoxide anion (O$_2^-$) and hydrogen peroxide in the biological systems, which in turn generate hydroxyl radicals, resulting in initiation and propagation of lipid peroxidation (Latha et al., 2003). The activity of super oxide dismutase was found to be lower in diabetic patients when compared to normal. This decrease in activity could result from activation of the enzyme by H$_2$O$_2$ or by glycation of the enzyme, which are known to occur during diabetes. Super oxide dismutase scavenges superoxide anion to form H$_2$O$_2$ and diminishes the toxic effects derived from secondary reaction. The activity of Superoxide dismutase was found to be lowered in diabetic controlled rats. Catalase is a haeme protein, which catalyses the reduction of hydrogen peroxides and protects the tissues from highly reactive hydroxyl radicals. This decrease in catalase activity could result from inactivation by glycation of the enzyme (Yoshida et al., 1995). The increase in SOD activity may indirectly play an important protective role in preserving the activity of catalase. The reduced activities of SOD and CAT in kidney have been observed during diabetes.

Glutathione peroxidase, an enzyme with selenium, works with glutathione in the decomposition of H$_2$O$_2$ or other organic hydroperoxides to non-toxic products at the expense of GSH. Reduced activities of glutathione peroxidase may result from radical induced inactivation and glycation of enzymes. Further, insufficient availability of GSH may also reduce the activity of GPx. Reduced activities of GPx in kidney have been observed during diabetes and this may result in a number of deleterious effects due to accumulation of toxic products. The MEAL and AEAL treatment increased the activity of enzymes (Table 1, Table 2) and may thereby help to control free radicals, as Aerva lanata Linn Juss has been reported to be rich in flavonoids, triterpenoids, well-known antioxidants and also to possess in vitro free radical scavenging and antioxidant activity (Appia Krishnan et al., 2009). Glutathione is a tripeptide normally present at high concentrations intracellularly, and constitutes the major reducing capacity of cytoplasm. Decreased level of GSH in kidney during diabetes represents its increased utilization due to oxidative stress (Wohaieb et al., 1987). GSH plays a pivotal role in the protection of cells against free radicals. Decreased GSH in hyperglycemia is due to decreased formation, GSH formation requires NADPH and glutathione reductase. Thus GSH is replenished by the administration of the effects of MEAL and AEAL on hexokinase, glucose-6-phosphatase, fructose - 1, 6 -bisphosphatase and liver glycogen in diabetic rats (Table 2). Flavanoids, glycosides stimulate the secretion of insulin in β- cells of pancreas (Hii et al., 1985). On the basis of above evidence it is possible that the presence of glycosides and tannins are responsible for the activity (Chauhan et al., 2007). Hepatic glycogen reserves are important for whole body glucose homeostasis and are markedly low in the diabetic state (Hornbrook., 1970; Migliorini 1971).

The gluconeogenic enzyme glucose - 6 - phosphatase is a crucial enzyme of glucose homeostasis because it catalyses the ultimate biochemical reaction of both glycolgenolysis and gluconeogenesis (Mithievre et al., 1996). These seem to be the consequence of the high glucose - 6 - phosphatase activities in a diabetic state (Defronza., 1998; Guignot et al., 1999). Glucose - 6 - phosphate dehydrogenase activity was decreased in diabetic state can
result in the diminished functioning of the pentose phosphate pathway and thereby the production of reducing equivalent such as NADH and NADPH (Weber et al., 1996). In the current study, the administration of MEAL and AEAL considerably increased the activity of glucose-6-phosphate dehydrogenase and decrease the activity of glucose-6-phosphatase, while the decrease in plasma glucose concentration causes the activation of the pentose phosphate pathway, inactivation of the sorbitol pathway and consequently an increase in the NADPH level (Sinclair., 1993). In the present study, the malondialdehyde (MDA) levels, a lipid peroxidation product and a marker of oxidative stress were elevated significantly in diabetic animals. Treatment with MEAL and AEAL significantly decreased the MDA levels (Table 3). In the current study, the liver glycogen concentration was significantly reduced in the diabetic rats due to disturbances in glycogen synthetase system. Improvement in liver glycogen after treatment with MEAL and AEAL may be due to improvement of glycogenesis or suppression of glycogenolysis (Table 3).

Since STZ causes selective destruction of β - cells of islets of Langerhans resulting in marked decrease in insulin levels, it is rational that glycogen levels in tissues (skeletal muscle and liver) decrease as they depend on insulin for influx of glucose (Whitten et al., 1975). The administration of MEAL and AEAL prevented the depletion of glycogen content but could not normalize it is due to the stimulation of insulin release from β - cells by Aerva lanata Linn Juss.

3 Material and Methods
3.1 Collection of plant materials
Fresh aerial parts of the plant Aerva lanata Linn Juss were collected during the month of November from Tirunelveli district in Tamil Nadu, India and it was identified and authenticated by Dr. Shiddamallayya N, Asst. Director in charge from Regional Research Institute (AY.), Bangalore. A Voucher specimen (RRCBI- 5588) was deposited in the Institute for future reference. The aerial parts of Aerva lanata Linn Juss were dried in the shade and it is milled into coarse powder by a mechanical grinder and it is stored in closed vessel for further use.

3.2 Preparation of plant extracts
The air dried coarse powder of the aerial parts of Aerva lanata was extracted successively with solvents of increasing polarity like petroleum ether, chloroform, acetone and methanol using soxhlet's apparatus and water by maceration for 7 days. The marc obtained from methanol extract was dried in air and soaked in water in a stoppered container and allowed to stand at room temperature for a period of atleast 3 days with frequent agitation until the soluble matter has dissolved. The mixture is then strained and the marc is pressed and the combined liquids are filtered. The marc was collected and dried in the air before extracting with next solvent; it is then packed in the soxhlet's apparatus. After each extraction was completed, the extracts were collected, filtered and concentrated under reduced pressure in the rotator evaporator. Finally it is dried and kept in the desiccators for further use.

3.3 Phytochemical screening
Phytochemical screening showed the presence of alkaloids, tannins, flavanoids and terpenoids (Kokate., 1994; Harborne., 1998).

3.4 Experimental animals
Male Sprague Dawley (150~180 g) rats were used for the present study. Rats were housed in polycarbonate cages in a room with a 12 hrs day-night cycle, at constant temperature of 22 C and humidity of 45~64%. The animals were fed with standard pellet diet and water ad libitum. The experimental protocol was approved by the Institutional animal ethics committee of Sri Ramachandra University, Chennai, India. Approval no IAEC- XII/ SRU/80. CPCSEA guidelines were adhered during the maintenance and experiment.

3.5 Acute toxicity study
Acute oral toxicity study of MEAL and AEAL was carried out in healthy rats (n=3) according to the guidelines framed by Organization for Economic Cooperation and development (OECD) guidelines (Bala et al., 2010).
Toxicity studies were carried out by giving the starting dose of 2000 mg/kg b.w. and finally a dose of 4000 mg/kg b.w. was given. The animals were observed every 1 hour and next 5-6 hours and daily thereafter for mortality, behavioural pattern changes such as weakness, aggressiveness, food or water refusal, diarrhea, salivation, discharge from eyes and ears, changes in locomotor activity, convulsion, coma, injury, pain or any sign of toxicity in each group of animals for a total of 14 days.

3.6 Experimental design
Rats were divided into seven groups of six rats (n=6) each. Group 1 served as vehicle control using normal saline (0.9% w/v NaCl), Group 2 served as diabetic control (Diabetes was induced in rats by intraperitoneal (i.p.) injection of STZ at a dose of 60 mg/kg body weight, dissolved in 0.1 M cold citrate buffer (pH= 4.5). Group 3 served as diabetic standard treated with 0.5 mg/kg of glibenclamide, p. o (micro labs), Group 4 and 5 was treated with MEAL 200 and 400 mg/kg, p.o., Group 6 and 7 was treated with AEAL 200 and 400 mg/kg, p.o. (Liu et al., 2008).

3.7 Preparation of tissue homogenate
The tissues were weighed and 10% tissue homogenate was prepared with 0.025 M Tris-HCl buffer, pH 7.5. After centrifugation at 1000 rpm for 10 min, the clear supernatant was used to measure thiobarbituric acid reactive substances (TBARS).

For the estimation of enzymatic antioxidants, tissues was minced and homogenized (10% w/v) in 0.1 M phosphate buffer (pH 7.0) and centrifuged at 1000 rpm for 10 min at 4°C. The supernatant obtained were used for enzyme assays.

3.8 Enzymatic bioassay
3.8.1 Lipid peroxidation
The method involved heating of biological samples with 0.8 ml saline, 0.5ml of BHT and 3.5 ml TBA reagent for 11/2 min in a boiling water bath. After cooling, the solution was centrifuged at 2,000 rpm for 10 min and the precipitate obtained was removed. The absorbance of the supernatant was determined at 532 nm using spectrophotometer against a blank that contained all the reagents minus the biological sample. The values were expressed in mg/ml serum (Okhawa et al., 1979).

3.8.2 Total protein by biuret method
Estimation of protein was assayed by taking 0.2 ml saline, 10% homogenate followed by addition of 1.25 ml of working biuret reagent. Incubated at room temp for 15 min. The colour intensity was read at 540 nm (Reinhold., 1953).

3.8.3 Catalase
The catalase activity was analyzed by the method of (Asru K Sinha, 1972) with slight modification. The reaction mixture contains 0.1 ml of the homogenate, 0.3 ml of H$_2$O$_2$ (2 mM) and 0.6 ml of phosphate buffer (10 mM, pH 7.4). The tubes were mixed well and heated for 5 min and then 2 ml of Dichromate Acetic Acid reagent (5% Potassium dichromate in water, Glacial acetic acid mixed in 1:3 ratio) was added to stop the reaction. Dichromate acetic acid reagent alone acts as blank. The intensity of color developed was read at 570 nm (Asru K Sinha., 1972).

3.8.4 Superoxide dismutase (SOD)
Superoxide dismutase was assayed by taking 0.05 ml of serum followed by addition of 0.3 ml of sodium pyrophosphate buffer (0.025 M, pH 8.3), 0.025 ml of PMS (186 µM) and 0.075 ml of NBT (300 µM in buffer of pH 8.3) The reaction was started by addition of 0.075 ml of NADH (780 µM in buffer of pH 8.3). After incubation at 30°C for 90 seconds, the reaction was stopped by addition of 0.25 ml glacial acetic acid. Then the reaction mixture was stirred vigorously and shaken with 2.0 ml of n-butanol. The mixture was allowed to stand for 10 minutes
and centrifuged. 1.5 ml of n-butanol alone was served as blank. The colour intensity of the chromogen was read at 560 nm (Kakkar et al., 1984).

3.8.5 Glutathione peroxidase (GPX)

Glutathione peroxidase (GPX) was assayed by taking 200 µl of tris HCL buffer (0.4 M), 0.4 mM K.EDTA along with 100 µl of sodium azide and 200 µl of enzyme preparation (hemolysate) and mixed well. Thereafter, 200 µl of reduced glutathione solution (2 mM) followed by 0.1 ml H₂O₂ were added. The overall reaction was arrested by adding 0.5 ml of 10% TCA. The precipitate was removed by centrifugation at 4000 rpm for 10 min. The absorbance was read at 412 nm using spectrophotometer. The non-enzymatic reaction rate was correspondingly assessed by replacing the enzyme sample by buffer. The results are expressed as nmoles/min/litre serum (Rotruck et al., 1973).

3.8.6 Reduced glutathione (GSH)

Glutathione content was estimated according to the method of (Ellman, 1959). 0.25 ml of serum was added to equal volume of ice cold 5% TCA. The precipitate was removed by centrifugation at 4000 rpm for 10 minutes. To 1 ml aliquot of supernatant, 0.25 ml of 0.2 M phosphate buffer, pH 8.0 and 0.5 ml of DTNB (0.6 mM in 0.2 M phosphate buffer, pH 8.0) was added and mixed well. The absorbance was read at 412 nm using spectrophotometer. The values were expressed in mg/dl serum (Moron et al., 1979).

3.8.7 Glucose-6 phosphatase

Glucose-6 Phosphatase was assayed by taking 0.3 ml of buffer followed by the addition of 0.5 ml of 0.01 M Glucose-6 Phosphatase as substrate. To the test, 0.2 ml of 10% homogenate was added and further incubated at 37°C for 1 hr. The reaction was immediately arrested by the addition of 10% TCA. The control reaction rate was correspondingly assessed by adding 0.2 ml of 10% homogenate only after the arresting step. The precipitate was removed by centrifugation at 3500 rpm for 10 minutes. To 50 µl of supernatant, 125 µl of distilled water, 125 µl of ammonium molybdate and 50 µl of ANSA was added and incubated for 10 mins at room temperature. The blue colour intensity was read immediately at 640 nm using spectrophotometer against a blank that contained all the reagents except the supernatant. The results are expressed in mcg/ mg of protein/ ml (Koida et al., 1959).

3.8.8 Fructose 1, 6 diphosphatase

Fructose 1, 6 diphosphatase was assayed by taking 1.2 ml of buffer followed by the addition of 0.1 ml of 0.005 M fructose 1, 6 diphosphate as substrate, 250 µl of MgCl₂, 0.1 ml of KCL and 0.25 ml of K. EDTA. To the test, 0.1 ml of 10% homogenate was added and further incubated at 37°C for 15 mins. The reaction was immediately arrested by the addition of 10% TCA. The control reaction rate was correspondingly assessed by adding 0.1 ml of 10% homogenate only after the arresting step. The precipitate was removed by centrifugation at 3500 rpm for 10 minutes. To 0.2 ml of supernatant, 0.8 ml of distilled water, 0.5 ml of ammonium molybdate and 0.2 ml of ANSA was added and incubated for 10 mins at 37°C. The blue colour intensity was read immediately at 640 nm using spectrophotometer against a blank that contained all the reagents minus the supernatant. The results are expressed in nmoles of Pi liberated /min/mg of protein (Gancedo et al., 1971).

3.8.9 Hexokinase

Hexokinase Activity was assayed by taking 2.5 ml of tris HCL buffer followed by the addition of 1 ml of 0.005 M glucose as substrate, 0.5 ml of 0.72 M ATP, along with 0.1 ml of 0.05 M MgCl₂, 0.1 ml of 0.5 M Sodium fluoride, 0.4 ml of 0.02 M KH₂PO₄ and 0.4 ml of 0.1 M KCl. The reaction mixture was pre- incubated at 37°C for 15 mins. Then 0.1 ml of 10% homogenate was added to the test alone and further incubated at 37°C for 30 mins. The reaction was immediately arrested by the addition of 10% TCA. The control reaction rate was correspondingly assessed by adding 0.1 ml of 10% homogenate only after the arresting reaction and the protein precipitate was removed by centrifugation at 3500 rpm for 10 minutes. To 0.2 ml of supernatant, 0.8 ml of distilled water and 4ml of anthrone was added and the absorbance was read at 630 nm using spectrophotometer. 1 ml
of buffer and 4 ml of anthrone alone served as blank. The results are expressed in nmoles of glucose consumed /min/mg of protein (Brandstrup et al., 1957).

3.8.10 Liver glycogen
100 μl of 10% homogenate was taken and to it 400 µl of 80% hot ethanol was added. This was centrifuged and washed for about two to three times. The residue obtained was dried in a sand bath for 2 - 3 mins. To the dried residue, 2 ml of distilled water and 2.5 ml of 52% perchloric acid was added and incubated at 0°C for 20 mins. The precipitate was removed by centrifugation at 3500 rpm for 10 mins. To 0.2 ml of supernatant, 0.8 ml of distilled water and 4ml of anthrone was added and boiled for 5 mins. The absorbance was read at 630 nm using spectrophotometer. 1 ml of distilled water and 4 ml of anthrone alone served as blank. The values were expressed in mg/dl serum (Seifter et al., 1950).

3.9 Statistical analysis
The results are expressed as mean ± S.E.M. Statistical difference was tested by using one-way analysis of variance (ANOVA) followed by Dunnet’s test. Values are expressed as mean ± SEM (n=6) in each group. **Values are significantly different from hyperglycemic control at p<0.01. * Values are significantly different from hyperglycemic control at p<0.05.

3.10 Conclusion
The aerial parts of Aerva lanata Lin Juss is a good candidate as alternative and/or complementary medicine in the management of diabetes mellitus. The results of the present study indicate that the MEAL at higher dose is capable of exhibiting potent antioxidant and lipid peroxidation activity can be employed in protecting tissue from the oxidative stress, which may be responsible for its antihyperglycemic activity. However, further investigations are needed to identify the lead molecule and to elucidate exact mechanism of action for antidiabetic activity.

Author’s contributions
All the authors contributed equally for this study. All the authors read and approved the final version of the manuscript.

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