Chapter 5
Chapter 5 Preliminary Pharmacological studies

5.1 Comparative evaluation of antihyperglycemic and antihyperlipidemic activity in streptozotocin induced diabetic rats

5.1.1. Introduction

Diabetes mellitus is a metabolic disorder characterized by disturbances in carbohydrate, protein and lipid metabolism and by complications like micro vascular (retinopathy, neuropathy and nephropathy) and macro vascular (heart attack, stroke and peripheral vascular disease) complications (Umar, 2010). A world wide survey has reported that diabetes mellitus affects nearly 10% of the population. It has been predicted that the prevalence of diabetes in adults will increase from 135 million in 1995 to 350 million in 2030 as given by International Diabetes Federation (Menaka, 2010 and Amos et al., 1997). Currently available synthetic antidiabetic agents produce serious side effects like hypoglycemic coma and hepatorenal disturbances (Sunil et al., 2009 and Kyriacou et al., 2010). Patients are therefore using herbal medicines which have fewer side effects and have the potential to impart therapeutic effect in complicated disorders like diabetes and its complication (Pandita et al., 2010). Following the WHO’s recommendation for research on the beneficial uses of medicinal plants in the treatment of diabetes mellitus, investigations on hypoglycemic agents derived from medicinal plants have also gained momentum. Traditional medicinal plants with various active principles and properties have been used from ancient times by physicians and laymen to treat a great variety of human diseases such as diabetes, coronary heart disease and cancer. Antidiabetic agents from medicinal plants could serve as a good source for drug design and much attention has been fixed on formulation of herbal medicine (Vishwakarma et al., 2010).

Insulin-resistant diabetes mellitus accounts for 90–95% of all diabetes. At present, therapy for type 2 diabetes relies mainly on several approaches intended to reduce the hyperglycaemia itself, sulphonylureas (and related insulin secretagogues), which increase insulin release from pancreatic islets; metformin, which acts to reduce hepatic glucose production; peroxisome proliferator-activated receptor-g (PPARg) agoniststhiazolidine -
diones, which enhance insulin action; α-glucosidase inhibitors, which interfere with gut glucose absorption; and insulin itself, which suppresses glucose production and augments glucose utilization. Characteristic factors include insulin resistance, obesity (in particular abdominal adiposity), hypertension, and a common form of dyslipidaemia (raised triglycerides and low high-density lipoprotein (HDL) cholesterol with or without elevation of low-density lipoprotein (LDL) cholesterol. Metabolic syndrome is associated with a markedly increased incidence of coronary, cerebral and peripheral artery disease. Thus, atherosclerotic cardiovascular disease (ASCVD) is responsible for 80% of diabetic mortality and more than 75% of all hospitalizations for diabetic complications. Indeed, type 2 diabetes now represents a coronary heart disease ‘risk equivalent’; this means that the risk of myocardial infarction in patients with diabetes and no history of cardiac disease roughly equates to the risk in non-diabetic patients with known cardiac disease (Haffner et al., 1998). Several mechanistic categories for new therapeutic approaches can be considered. First are approaches aimed at reducing excessive glucose production by the liver; second, mechanisms to augment glucose-stimulated insulin secretion; third, specific molecular targets in the insulin signalling pathway; and fourth, new approaches to obesity and altered lipid metabolism, which offer the prospect of net improvements in insulin action (or secretion).

- **Reducing excessive hepatic glucose production**

The liver has a critical role in regulating endogenous glucose production (gluconeogenesis) or the catabolism of glycogen (glycogenolysis). A relative decrease in insulin levels, or reduced hepatic responsiveness to insulin, can lead to increased output of glucose by the liver. Several drug targets in the liver offer new ways of attenuating excessive hepatic glucose production (DeFronzo et al., 1992). Glucagon is a well described hormone that contributes to hyperglycaemia through the induction of both gluconeogenic and glycogenolytic pathways (Shah et al., 2000). Other hepatic enzyme targets that have received more limited attention include fructose-1, 6-bisphosphatase and glucose − 6-phosphatase. Inhibition of the former would selectively block gluconeogenesis by disrupting
the conversion of fructose – 1,6-bisphosphate to fructose – 6-phosphate (Zhang and Moller, 2000).

- **Enhancing glucose-stimulated insulin secretion**

  A key component of the pathophysiology of type 2 diabetes involves a relatively selective defect in the ability of glucose to provoke secretion of insulin from pancreatic islet β-cells. This defect accounts for the failure of the β-cell to compensate for increasing insulin resistance and for the ultimate development of overt hyperglycaemia. Unlike sulphonylureas and related compounds, which stimulate insulin secretion in the absence of high glucose levels and work by blocking ATP-sensitive K+ channels, more desirable alternative approaches would potentiate insulin secretion in a purely glucose-dependent fashion. In this regard, two distinct gut-derived peptide hormones glucagon-like peptide-1 (GLP-1) and gastric inhibitory peptide (GIP) act through their respective G-protein-coupled receptors on b-cells to potentiate glucose-stimulated insulin secretion (Drucker, 2001).

- **Targeting the insulin signalling pathway**

  The role of peripheral and hepatic insulin resistance in the pathogenesis of diabetes is undisputed. Insulin resistance can be due to multiple defects in signal transduction (such as impaired activation of insulin receptor-tyrosine kinase and reduced activation of insulin-stimulated phosphatidylinositol – 3- OH kinase. A number of molecular targets are now being investigated as ways of enhancing insulin-mediated signal transduction. Non-peptide small molecules that can activate the insulin receptor, or potentiate its activation by insulin, have proved elusive. But the recent discovery of a small-molecule natural-product derivative that mediates selective activation of the insulin receptor is encouraging. An alternative approach to targeting the insulin receptor itself would be to inhibit enzymes responsible for deactivation of the receptor or downstream targets in the signalling pathway (for example, IRS proteins).

- **Targeting obesity, lipid metabolism and ‘lipotoxicity’**

  Abnormalities of fatty-acid metabolism are increasingly recognized as key components of the pathogenesis of the metabolic syndrome and type 2 diabetes. Fat-feeding and raised levels
of circulating free fatty acids (FFAs) are clearly sufficient to induce peripheral and hepatic insulin resistance. Accumulation of lipids inside muscle cells and specific increases in muscle long-chain fattyacyl – CoA content has been implicated in causing insulin resistance. In addition, lipid accumulation within pancreatic islets has been proposed to impair insulin secretion. These observations support a unified ‘lipotoxicity’ hypothesis, which states that metabolic syndrome and type 2 diabetes can be caused by the accumulation of triglycerides and long-chain fatty-acyl-CoA in liver and muscle (leading to a reduction in insulin-mediated metabolic activity) and in the islet (leading to impaired insulin secretion).

Type 1 diabetes is an autoimmune disease resulting from specific destruction of the insulin producing β-cells of the islets of Langerhans of the pancreas (Tisch and McDevitt, 1996). It has two distinct phases: insulitis, when a mixed population of leukocytes invades the islets; and diabetes, when most β-cells have been killed off, and there is no longer sufficient insulin production to regulate blood glucose levels, resulting in hyperglycaemia. Individuals can have covert insulitis for a long time (years in humans, months in rodent models) before it finally progresses to overt diabetes, and sometimes it never does.

Type 1 diabetes is an old disorder - descriptions of it appear in ancient Egyptian and Greek writings. It is also a common disease, currently affecting about 0.5% of the population in developed countries and increasing in incidence. In addition, there is mounting evidence that a fraction (variously estimated at 5–15%) of people originally diagnosed as type 2 diabetic may actually have a slowly progressing and less severe form of type 1 termed ‘latent autoimmune diabetes of adults’. The second forms of diabetes are characterized by chronic hyperglycaemia and the development of diabetes-specific micro vascular pathology in the retina, renal glomerulus and peripheral nerve. As a consequence of its microvascular pathology, diabetes is a leading cause of blindness, end stage renal disease and a variety of debilitating neuropathies. Diabetes is also associated with accelerated atherosclerotic macro vascular disease affecting arteries that supply the heart, brain and lower extremities. As a result, patients with diabetes have a much higher risk of myocardial infarction, stroke and limb amputation. Large prospective clinical studies show a strong relationship between
glycaemia and diabetic micro vascular complications in both type 1 and type 2 diabetes. Hyperglycaemia and insulin resistance both seem to have important roles in the pathogenesis of macro vascular complications (Wei et al, 1998; Ebara, 2000; Ginsberg, 2000). The pathogenesis of atherosclerosis in non-diabetics has been extensively described in recent reviews, and begins with endothelial dysfunction (Lusis, 2000). In diabetic arteries, endothelial dysfunction seems to involve both insulin resistance specific to the phosphatidylinositol -3-OH kinase pathway (Hsueh and Law, 1998; Jiang, 1999) and hyperglycaemia. Postprandial hyperglycaemia may be more predictive of atherosclerosis than is fasting plasma glucose level or haemoglobin A1C (Temelkova-Kurktschiev, 2000).

**Mechanism of hyperglycemia induced damage**

Four main hypotheses about how hyperglycaemia causes diabetic complications have generated a large amount of data, as well as several clinical trials based on specific inhibitors of these mechanisms. The four hypotheses are: increased polyol pathway flux; increased advanced glycation end-product (AGE) formation; activation of protein kinase C (PKC) isoforms; and increased hexosamine pathway flux. Until recently there was no unifying hypothesis linking these four mechanisms.

- **Increased polyol pathway flux**

  A number of mechanisms have been proposed to explain the potential detrimental effects of hyperglycaemia-induced increases in polyol pathway flux. These include sorbitol-induced osmotic stress, decreased (Na+ and K+) ATPase activity, an increase in cytosolic NADH/NAD+ and a decrease in cytosolic NADPH. Sorbitol does not diffuse easily across cell membranes, and it was originally suggested that this resulted in osmotic damage to microvascular cells. Sorbitol concentrations measured in diabetic vessels and nerves are, however, far too low to cause osmotic damage. It has also been proposed that reduction of glucose to sorbitol by NADPH consumes NADPH. As NADPH is required for regenerating reduced glutathione (GSH), this could induce or exacerbate intracellular oxidative stress. Decreased levels of GSH have in fact been found in the lenses of transgenic mice that over
express aldose reductase and this is the most likely mechanism by which increased flux through the polyol pathway has deleterious consequences (Lee and Chung, 1999).

- **Increased intracellular formation of advanced glycation end-products**
  AGEs are found in increased amounts in diabetic retinal vessels and renal glomeruli. They were originally thought to arise from nonenzymatic reactions between extracellular proteins and glucose. AGEs can arise from intracellular auto-oxidation of glucose to glyoxal. The potential importance of AGEs in the pathogenesis of diabetic complications is indicated by the observation in animal models that two structurally unrelated AGE inhibitors partially prevented various functional and structural manifestations of diabetic microvascular disease in retina, kidney and nerve (Soulis-Liparota et al., 1991).

- **Activation of protein kinase C**
 Activation of PKC-b iso forms has been shown to mediate retinal and renal blood flow abnormalities, perhaps by depressing nitric oxide production and/or increasing endothelin-1 activity. Abnormal activation of PKC has been implicated in the decreased glomerular production of nitric oxide induced by experimental diabetes, and in the decreased production of nitric oxide in smooth muscle cells that is induced by hyperglycaemia (Ganz and Seftel, 2000).

- **Increased flux through the hexosamine pathway**
  Shunting of excess intracellular glucose into the hexosamine pathway might also cause several manifestations of diabetic complications (Kolm-Litty et al., 1998). This pathway is also important role in hyperglycaemia induced and fat-induced insulin resistance (Marshall, 1991).

- **A common element linking hyperglycaemia-induced damage**
  Although specific inhibitors of aldose reductase activity, AGE formation, PKC activation and the hexosamine pathway each ameliorate various diabetes-induced abnormalities in cell culture and animal models, there has been no apparent common element linking the four mechanisms of hyperglycaemia-induced damage (Lee et al., 1995). This issue has now been resolved by the recent discovery that each of the four different pathogenic mechanisms
reflects a single hyperglycaemia-induced process: overproduction of superoxide by the
mitochondrial electron-transport chain. Many studies have shown that diabetes and
hyperglycaemia increase oxidative stress, but neither the underlying mechanism nor the
consequences for other pathways of hyperglycaemic damage were known (Giugliano et al.,
1996)

5.1.2 MATERIALS AND METHODS

5.1.2.1 Chemicals and reagents
Streptozotocin (Sigma chemical co., U.S.A), glibenclamide (micro labs, India), glucose,
triglyceride and total cholesterol, plasma biochemical parameters estimation kit (Accurex
Biomedical pvt Ltd, India). Other chemicals and reagents used for the study were of
analytical grade and procured from approved organizations.

5.1.2.2 Experimental animals
Male Sprague Dawley (150 – 180gms) rats were used for the present study. The animals
were maintained under standard environmental conditions and were fed with standard pellet
diet and water ad libitum. The experimental protocol was approved by the Institutional animal
ethics committee, Approval no IAEC- XII/ SRU/80/ 2008. CPCSEA guidelines were adhered
during the maintenance and experiment.

5.1.2.3 Acute toxicity studies
Acute oral toxicity study of MEAL and AEAL was conducted according to the guidelines set
by Organisation for Economic Cooperation and Development (OECD) guidelines (Bala et al,
2010). Young healthy adult Sprague dawley female rats weighing between 160 -180g b. wt,
were divided into two groups of 3 animals/group. Animals were housed individually in
a well ventilated polypropylene cage. A 12-h light/ 12-h dark artificial photoperiod was
maintained. Room temperature 22°C (± 3°) and relative humidity 50–70 % were maintained
in the room. Animals had free access to pelleted feed. (Nutrilab rodent, Tetragon Chemie Pvt
Ltd., India) and Reverse osmosis (Rios, USA) purified water ad libitum. The extracts were
administered once orally via gastric intubation at a dose level of 2000 mg/ kg b.wt. Lethality,
abnormal clinical signs and body weight changes were observed on the day of dosing and
thereafter for 13 days. Gross pathological changes were also observed on the termination of the experiment.

5.1.2.4 Evaluation of anti diabetic activity

a) Treatment protocol

The animals were divided into seven groups of six animals each as follows

Group I- Vehicle control, Normal saline (0.9 % w/v NaCl).

Group II- Diabetic control

Group III- Diabetic standard treated, 0.5 mg/ kg of glibenclamide, p. o (micro labs)

Group IV- Diabetes MEAL 200 mg/ kg, p.o.

Group V- Diabetes MEAL 400 mg/ kg, p.o.

Group VI- Diabetes AEAL 200 mg/ kg, p.o.

Group VII- Diabetes AEAL 400 mg/ kg, p.o.

Diabetes was induced in all groups except normal control by a single intraperitoneal injection of 60 mg/ kg of Streptozotocin (STZ) dissolved in a freshly prepared 0.1 M citrate buffer (pH 4.5). The animals in the vehicle control (Group I) received normal saline orally (0.9 % w/ v NaCl). The rats with blood glucose levels above 250 mg/ dl were considered as diabetic and used in this study (Liu et al., 2008). After 72 h, the blood was withdrawn by retro orbital puncture under light ether anaesthesia and the blood glucose level was estimated. Serum was separated by centrifugation at 3000 rpm for about 5 mins. The clear straw coloured serum was collected and stored at 4°C for the measurement of marker enzymes level to assess the liver functions. Blood glucose levels and body weight were measured on day 0, 7 and 14 of the study. Finally on day 14, blood was collected to perform various biochemical (Anreddy et al., 2010) enzymatic and antioxidant profile was studied.

b) Estimation of MEAL and AEAL on fasting blood glucose

The plasma glucose estimation was carried out based on enzymatic method using glucose oxidase/ peroxidase (GOD/ POD) method (Abdel-Barry et al., 1997) using a standard kit obtained from Accurex Biomedical pvt Ltd, India. Serum glucose levels are expressed in mg/ dl. Glucose is oxidized by glucose oxidase (GOD) to produce gluconate and hydrogen
peroxide. The hydrogen peroxide is then oxidatively coupled with 4 amino-antipyrine (4-AAP) and phenol in the presence of peroxidase (POD) to yield a red quinoneimine dye that is measured at 505 nm. The absorbance at 505 nm is proportional to concentration of glucose in the sample.

\[
\begin{align*}
\text{Glucose} + 2 \text{H}_2\text{O} + \text{O}_2 & \xrightarrow{\text{GOD}} \text{Gluconate} + \text{H}_2\text{O}_2 \\
2 \text{H}_2\text{O}_2 + 4\text{-AAP} + \text{Phenol} & \xrightarrow{\text{POD}} \text{Quinoneimine dye}
\end{align*}
\]

Absorbance of the colored solution was directly proportional to the glucose concentration, when measured at 505 nm.

Every week, following overnight fasting (16 h fasting with free access to water), the blood samples were withdrawn from the animals by retro orbital puncture under light ether anaesthesia.

- **Components and concentration of working solution**

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphate buffer, pH 7.0</td>
<td>170 mmol/ l</td>
</tr>
<tr>
<td>Glucose oxidase</td>
<td>15000 IU/ l</td>
</tr>
<tr>
<td>Peroxidase</td>
<td>1500 IU/ l</td>
</tr>
<tr>
<td>4-aminoantipyrine</td>
<td>0.28 mmol/ l</td>
</tr>
<tr>
<td>Phenol</td>
<td>16 mmol/ l</td>
</tr>
</tbody>
</table>

- **Procedure**

The assay was performed as given below

1.0 ml of procedure

<table>
<thead>
<tr>
<th>Serum/plasma</th>
<th>Standard</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01 ml</td>
<td>0.01 ml</td>
<td>-</td>
</tr>
<tr>
<td>1.0 ml</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
</tr>
</tbody>
</table>

The assay mixture was incubated for 7 minutes at 37°C for 15 mins at room temperature (25°C- 30°C). After completion of incubation period, the absorbance was measured against blank at 505 nm. Final colour was stable for two hours if not exposed to direct light.
Glucose (mg/dl) = \frac{\text{Abs of sample}}{\text{Abs of standard}} \times \text{conc. of standard (mg/dl)}

c. Effect of MEAL and AEAL on total cholesterol (TC) levels

Cholesterol is measured enzymatically in serum or plasma in a series of coupled reactions that hydrolyze cholesteryl esters and oxidize the 3–OH group of cholesterol. One of the reaction byproducts, \( \text{H}_2\text{O}_2 \) is measured quantitatively in a peroxidase catalyzed reaction that produces a colour. Absorbance is measured at 500 nm. The colour intensity is proportional to cholesterol concentration. The reaction sequence is as follows:

\[
\text{Cholesteryl ester} + \text{H}_2\text{O}_2 \xrightarrow{\text{Cholesterol ester hydrolase}} \text{Cholesterol} + \text{fatty acid}
\]

\[
\text{Cholesterol} + \text{O}_2 \xrightarrow{\text{Cholesterol oxidase}} \text{Cholester-4-en-3-one} + \text{H}_2\text{O}_2
\]

\[
2 \text{H}_2\text{O}_2 + 4\text{- amino phenazone} + \text{phenol} \xrightarrow{\text{Peroxidase}} 4\text{- (p-benzoquinone-monoamino)-phenazone} + 4 \text{H}_2\text{O}
\]

- **Components and concentration of working solution**

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer, pH 6.8</td>
<td>50 mmol/l</td>
</tr>
<tr>
<td>Cholesterol oxidase</td>
<td>≥ 100 IU/l</td>
</tr>
<tr>
<td>Cholesterol esterase</td>
<td>≥ 150 IU/l</td>
</tr>
<tr>
<td>Peroxidase</td>
<td>≥ 500 IU/l</td>
</tr>
<tr>
<td>4-amino antipyrine</td>
<td>0.5 mmol/l</td>
</tr>
<tr>
<td>Phenol</td>
<td>≥ 10 mmol/l</td>
</tr>
</tbody>
</table>

- **Procedure**

The required amount of reagent was prewarmed at room temperature. The assay was performed as given below.

<table>
<thead>
<tr>
<th>Serum</th>
<th>Standard</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01 ml</td>
<td>0.01 ml</td>
<td>-</td>
</tr>
<tr>
<td>1.0 ml</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
</tr>
</tbody>
</table>
The assay mixture was incubated for 5 mins at 37°C or 10 minutes at room temperature (25 - 30°C). After incubation, the absorbance of assay mixture was measured against blank at 510 nm. The final colour was stable for two hours if not exposed to direct light.

\[
\text{Conc. (mg/dl)} = \frac{\text{Abs of sample}}{\text{Abs of standard}} \times 200
\]

**d. Effect of MEAL and AEAL on serum triglycerides (TG)**

Triglycerides are measured enzymatically in serum or plasma using a series of coupled reactions in which triglycerides are hydrolyzed to produce glycerol. Glycerol is then oxidized using glycerol oxidase, and \( \text{H}_2\text{O}_2 \), one of the reaction products, is measured as described above for cholesterol. Absorbance is measured at 550 nm. The reaction sequence is as follows:

\[
\text{Triglycerides} + 3 \text{H}_2\text{O} \xrightarrow{\text{Lipase}} \text{glycerol} + \text{Fatty acids}
\]

\[
\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerokinase}} \text{Glycerol-3-phosphate} + \text{ADP}
\]

\[
\text{Glycerol-3-phosphate} + \text{O}_2 \xrightarrow{\text{glycerophosphate oxidase}} \text{Dihydroxyacetone phosphate} + \text{H}_2\text{O}_2.
\]

\[
\text{H}_2\text{O}_2 + 4\text{-aminophenazone} + 4\text{-chlorophenol} \xrightarrow{\text{peroxidase}} 4\text{-}(p\text{-benzoquinone-monoimino)}\text{-phenazone} + 2\text{H}_2\text{O} + \text{Hcl}
\]

- Components and concentration of working solution

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer, pH 7.2</td>
<td>50 mmol/l</td>
</tr>
<tr>
<td>Lipase</td>
<td>≥ 2000 IU/l</td>
</tr>
<tr>
<td>Glycerol kinase</td>
<td>≥ 300 IU/l</td>
</tr>
<tr>
<td>Glycerol phosphate oxidase</td>
<td>≥ 1000 IU/l</td>
</tr>
<tr>
<td>Peroxidase</td>
<td>≥ 500 IU/l</td>
</tr>
<tr>
<td>ATP</td>
<td>1 mmol/l</td>
</tr>
<tr>
<td>Chromogen</td>
<td>2 mmol/l</td>
</tr>
</tbody>
</table>
• Procedure

The required amount of reagent was prewarmed at room temperature (25°C - 30°C).

• 1 ml of procedure

<table>
<thead>
<tr>
<th>Serum/ Plasma</th>
<th>Standard</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01 ml</td>
<td>0.01 ml</td>
<td>-</td>
</tr>
<tr>
<td>1.0 ml</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
</tr>
</tbody>
</table>

The assay mixture was incubated for 10 mins at 37°C for 20 mins at room temperature (25°C - 30°C). After incubation, the absorbance was measured against blank at 510 nm (500 - 530 nm). Final colour was stable for 30 mins if not exposed to direct light.

\[
\text{Conc. (mg %)} = \frac{\text{Abs of sample}}{\text{Abs of standard}} \times 200
\]

e. Effect on body weight

During the study period of 14 days the mice were weighed daily and the body weight of all experimental animals was recorded using a digital weighing scale and SEM were calculated and tabulated (Salahuddin et al., 2010).

f. Biochemical estimation

The effect of MEAL and AEAL treatment on the biochemical parameters of the experimental rats were evaluated by the estimation of serum biochemical enzymes such as,

i. Serum glutamic oxaloacetic transaminase (SGOT) (Reitman and Frankel, 1957)

• In this reaction L- aspartate and α- ketoglutarate react in the presence of GOT in the sample to yield oxaloacetate and L-glutamate.

\[
\text{GOT (AST)}
\]

\[
\text{L- Aspartate + α- ketoglutarate} \rightarrow \text{oxaloacetate + L- glutamate}
\]

• The oxaloacetate is reduced by malate dehydrogenase (MDH) to yield L-malate with the oxidation of NADH to NAD⁺. The reaction is monitored by measurement of the decrease in absorbance of NADH at 340 nm.
Oxaloacetate + NADH + H⁺ → L-malate + NAD⁺

The conversion of NADH to NAD⁺ is proportional to the concentration of GOT in serum and is measured at 340 nm, as rate of decrease in absorbance.

- **Components and concentration of working solution**

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris buffer, pH 7.8</td>
<td>80 mmol/ l</td>
</tr>
<tr>
<td>L-aspartate</td>
<td>240 mmol/ l</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>≥ 3000 IU/ l</td>
</tr>
<tr>
<td>Malate dehydrogenase</td>
<td>≥ 400 IU/ l</td>
</tr>
<tr>
<td>NADH</td>
<td>0.23 mmol/ l</td>
</tr>
<tr>
<td>α-ketoglutarate</td>
<td>10 mmol/ l</td>
</tr>
</tbody>
</table>

- **Procedure**

The required amount of working solution was prewarmed at the required temperature 37°C.

The assay was performed as given below

1.0 ml of procedure

Serum/ Plasma - 0.05 ml

Working solution - 1.0 ml

Mix thoroughly and the assay mixture was transferred immediately to the thermostated cuvette and stopwatch was started simultaneously. First reading was recorded at 60th second and subsequently three more readings was recorded with 30 seconds interval at 340 nm.

- **Calculation**

Calculate the change in absorbance per minute

\[(Δ \text{Abs/ 30 seconds } \times 2)\]

Activity of GOT (AST) in IU/l = \[Δ \text{Abs/ min } \times 3339\]
ii. Serum glutamic pyruvic transaminase (SGPT) (Reitman and Frankel, 1957)

α-ketoglutarate reacts with L-alanine in presence of GPT (ALT) to form pyruvate and L-glutamate. The increase in pyruvate is determined in an indicator reaction catalyzed by lactate dehydrogenase. The conversion of NADH to NAD$^+$ at 340 nm is proportional to the activity of GPT (ALT) in serum/plasma and is determined kinetically as rate of decrease in absorbance.

\[
\text{L-Alanine + } \alpha\text{-ketoglutarate } \xrightarrow{\text{GPT (ALT)}} \text{pyruvate + L-glutamate} \\
\text{Pyruvate + NADH + H}^+ \xrightarrow{\text{Lactate dehydrogenase}} \text{L-lactate + NAD}^+
\]

- Components and concentration of working solution

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris buffer, pH 7.4</td>
<td>80 mmol/l</td>
</tr>
<tr>
<td>L-alanine</td>
<td>500 mmol/l</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>≥ 3000 IU/l</td>
</tr>
<tr>
<td>NADH</td>
<td>0.23 mmol/l</td>
</tr>
<tr>
<td>α-ketoglutarate</td>
<td>10 mmol/l</td>
</tr>
</tbody>
</table>

- Procedure

The required amount of working solution was prewarmed at the required temperature 37°C.

The assay was performed as given below

- 1 ml of procedure

<table>
<thead>
<tr>
<th>Serum/ Plasma</th>
<th>0.02 ml (20 μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working solution</td>
<td>1.0 ml</td>
</tr>
</tbody>
</table>

Mix thoroughly and the assay mixture was transferred immediately to the thermostated cuvette and stop watch was started simultaneously. The first reading at 60th second and subsequently three more readings with 30 seconds interval at 340 nm was recorded.
• Calculation

Calculate the change in absorbance per minute

\( \Delta \text{Abs/ 30 seconds} \times 2 \)

Activity of GOT (AST) in IU/ l = \( \Delta \text{Abs/ min} \times 3339 \)

• Alkaline phosphatase (ALP) (Kind and King, 1954)

ALP cleaves p-nitrophenyl phosphate into p-nitrophenol and phosphate. P-nitrophenol is a yellow colour compound in alkaline medium and absorbs light at 405 nm. The rate of increase in absorbance at 405 nm is proportional to alkaline phosphatase activity in specimen.

\[
\text{Alk. Phos}
\]

\[
P\text{-nitrophenyl phosphate} \rightarrow p\text{-nitrophenol + phosphate}
\]

• Components and concentration of working solution

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diethanolamine</td>
<td>1 mol/ l</td>
</tr>
<tr>
<td>p- nitrophenyl phosphate</td>
<td>10 mmol/ l</td>
</tr>
<tr>
<td>Magnesium chloride</td>
<td>0.5 mmol/ l</td>
</tr>
</tbody>
</table>

• Procedure

The required amount of working solution was prewarmed at the required temperature 37\(^\circ\) C.

The assay was performed as given below

• 1.0 ml of procedure

<table>
<thead>
<tr>
<th>Serum/ Plasma</th>
<th>0.02 ml (20 (\mu l))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working solution</td>
<td>1.0 ml</td>
</tr>
</tbody>
</table>

The assay mixture was mixed thoroughly and transferred immediately to the thermostated cuvette and the stop watch was started simultaneously. The first reading at 60\(^\text{th}\) second and subsequently, three more readings with 30 seconds interval at 405 nm was recorded.
• Calculation

Calculate the average change in absorbance per minute

Alkaline Phosphatase (IU/ I) = Δ Abs/ min × 2720

iv. Creatinine (Bartels et al., 1972)

Creatinine in alkaline medium reacts with picrate to produce orange colour. This colour absorbs light at 492 nm (490 - 510 nm). The rate of increase in absorbance is directly proportional to the concentration of creatinine in specimen.

Creatinine + Picrate → Alkaline medium → Orange colour

• Components and concentration of working solution

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium picrate</td>
<td>7.7 mmol/ l</td>
</tr>
<tr>
<td>Sodium hydroxide</td>
<td>500 mmol/ l</td>
</tr>
</tbody>
</table>

• 1.0 ml procedure

Standard / Sample - 0.05 ml
Working solution - 1.0 ml

Mixed and stopwatch was started simultaneously. Absorbance of assay mixture was recorded at exactly 30 seconds after standard/ specimen addition and then at 90 seconds.

• Calculation

Calculate the average change in absorbance per minute (Δ Abs) of standard and specimen

Δ Abs = Abs at 90 sec – Abs at 30 sec

Serum creatinine (mg %) = \[
\frac{\Delta \text{ Abs of specimen}}{\Delta \text{ Abs of standard}} \times 2
\]

v. Blood Urea Nitrogen (Bun) (Kaplan, 1965)

Urea is hydrolysed to ammonia and carbondioxide by urease. Ammonia produced reacts with α-ketoglutarate to form glutamate in presence of glutamate dehydrogenase. NADH is oxidised to NAD⁺ in this reaction, which is measured as decrease in absorbance at 340 nm.
The rate of decrease in absorbance at 340 nm is directly proportional to BUN concentration in the specimen.

\[
\text{Urease} \\
\text{Urea} + \text{H}_2\text{O} \rightarrow 2 \text{NH}_3 + \text{CO}_2
\]

\[
\text{NH}_3 + \alpha\text{-ketoglutarate} + \text{NADH} \rightarrow \text{glutamate} + \text{NAD}^+
\]

- Components and concentration of working solution

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris buffer, pH 7.6</td>
<td>120 mmol/ l</td>
</tr>
<tr>
<td>Urease</td>
<td>( \geq 20 ) KU/ l</td>
</tr>
<tr>
<td>Glutamate dehydrogenase</td>
<td>( \geq 1 ) KU/ l</td>
</tr>
<tr>
<td>NADH</td>
<td>0.25 mmol/ l</td>
</tr>
<tr>
<td>A-ketoglutarate</td>
<td>10 mmol/ l</td>
</tr>
</tbody>
</table>

- Procedure

The required amount of working solution was prewarmed at 25\(^0\) C / 30\(^0\) C. The assay was performed as given below

- 1ml of procedure

Standard/ Serum/ Plasma – 0.01 ml

Working solution – 1.0 ml

The assay mixture was mixed thoroughly and transferred immediately to the thermostated cuvette and stop watch was started simultaneously. The first reading at 30\(^{th}\) seconds and subsequently one more reading with 30seconds interval at 340 nm was recorded.

- Calculation

Calculate the change in absorbance (\(\Delta\) Abs) of standard and specimen

Factor = Concentration of standard + \(\Delta\) Abs of standard = 20 ÷ \(\Delta\) Abs. of Standard

Concentration of BUN in mg/ dl = \(\Delta\) Abs of Specimen × Factor
vi. **Total bilirubin** (Mallay and Evelyn, 1937)

Bilirubin reacts with diazotized sulphanilic acid to produce azobilirubin (violet colour). DMSO catalyzes the formation of azobilirubin from free bilirubin. The violet colour is proportional to bilirubin concentration measured at 546 nm (530 – 550 nm)

\[
\text{Total Bilirubin} + \text{Sulphanilic acid} + \text{Sodium Nitrite} \xrightarrow{\text{DMSO}} \text{Azobilirubin}
\]

- **Components and concentration of working solution**
  - Component - Concentration (Total Bilirubin)
    - Sulphanilic acid - 32 mmol/ l
    - Sodium nitrite - 290 mmol/ l
    - Hydrochloric acid - 165 mmol/ l
    - DMSO - 7 mmol/ l

The assay mixture was mixed and incubated at room temperature (25 - 30°C) for 5 mins. The absorbance of the test against their respective blanks at 546 nm (530 -550 nm) was recorded.

**Calculation**

\[
\text{Bilirubin mg %} = (\text{Abs. of Test} - \text{Abs. of Blank}) \times 20.2
\]

vii. **Total protein by Biuret method** (Reinhold, 1953)

Proteins react with cupric ions under alkaline pH to produce a colour complex. This colour complex absorbs light at 546 nm (530-570 nm). The intensity of the colour is directly proportional to the protein concentration in the specimen.

\[
\text{Proteins} + \text{Cu}^{2+} \rightarrow \text{Blue colour complex (Alkaline complex)}
\]

- **Components and concentration of working solution**
  - Component - Concentration
    - Cupric sulphate - 7 mmol/ l
    - Potassium iodide - 6 mmol/ l
    - Tartarate - 20 mmol/ l
• Procedure

The required amount of working solution was prewarmed at room temperature (25 - 30°C).

1.0 ml procedure

Serum/ Plasma – 0.01 ml

Standard – 0.01 ml

Working solution – 1.0 ml

The assay mixture was incubated for 15 mins at 37°C. After completion of incubation period, the absorbance of specimen and standard against blank was measured.

Calculation

\[
\text{Total protein gm \%} = \frac{\text{Abs of sample}}{\text{Abs of standard}} \times 6
\]

viii. 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 H₂O₂ (2 mM) and 0.6 ml of phosphate buffer (10 mM, pH 7.4). The tubes were mixed well and incubated at 37°C 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 using Thermo scientific multiskan spectrophotometer, USA.

ix. 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).
x. **Lipid peroxidation (TBARS)**

The method involved heating of biological samples with 0.8 ml saline, 0.5 ml of BHT and 3.5 ml of TBA reagent for 11/2 min in a boiling water bath. After cooling, the solution was centrifuged at 2000 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 (Ohkawa et al., 1979).

xi. **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 mins. 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 mcg/ mg/ prtn/ ml (Rotruck et al., 1973).

xii. **Reduced glutathione (GSH)**

Glutathione content was estimated according to the method of (Ellman, 1959). 0.25ml of serum was added to equal volume of ice cold 5% TCA. The precipitate was removed by centrifugation at 4000 rpm for 10 mins. To 1 ml aliquot of supernatant, 0.25 ml of 0.2 M phosphate buffer, pH 8.0 and 0.5ml of DTNB (0.6mM in 0.2M 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.

xiii. **Glucose -6 - phosphatase**

Glucose-6 phosphatase was assayed by the method of 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, 1075 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 minus the supernatant. The results are expressed in mcg/ mg/ prtn/ ml (Koida and Oda, 1959).

xiv. **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 reactions 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 mcg/ mg/ prtn/ ml (Gancedo and Gancedo, 1971).

xv. **Hexokinase activity**

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 mins.
To 0.2 ml of supernatant, 0.8 ml of distilled water and 4 ml 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 mcg/mg/prtn/ml (Brandstrup et al., 1957).

xvi. **Liver glycogen estimation**

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 4 ml of anthrone was added and boiled for 5 mins. The absorbance was read at 630 nm using spectrophotometer. 1ml of distilled water and 4 ml of anthrone alone served as blank. The values were expressed in mg/dl serum (Seifter et al., 1950).

xvii. **Histopathology**

On Day 14 after the completion of experiment, the animals were fasted overnight. All animals from each group were sacrificed by cervical dislocation under general anaesthesia. The brain, eyes, heart, liver, kidneys and pancreas from all the female rats were examined for gross pathological findings. The organs were collected and fixed in 10 % neutral buffered formalin for 48 hours, processed for paraffin embedment, sectioned and stained with hematoxylin and eosin for histopathological evaluation.

**5.1.2.5 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.**
5.1.3 Results

5.1.3.1 Acute toxicity study

The various observations showed the normal behaviour of the treated rats. No toxic effects were observed at a higher dose of 4 g/ kg body weight. There were no treatment related deaths, abnormal clinical signs, remarkable body weight changes or gross pathological changes were observed in the experimental animals. From the above results, LD$_{50}$ of the test drug was found to be greater than 2000 mg/ kg b.wt. Hence, the test drug falls in the “Category-5” or “unclassified” in accordance to the globally harmonised System.

5.1.3.2 Effect of AEAL and MEAL on fasting blood glucose

Treatment with methanol and aqueous extracts of aerial parts of Aerva lanata Linn Juss at the dose of 200 and 400 mg/ kg body weight for 1$^{st}$ and 2$^{nd}$ week exhibited a significant (p < 0.01) decrease in the fasting blood glucose in streptozotocin induced diabetic animals as compared to diabetic control. Blood glucose level of diabetic animals started decreasing from the first week of drug treatment that was continued to maintain till 2$^{nd}$ week, which was comparable to glibenclamide 0.5 mg/ kg (Table no. 31).

5.1.3.3 Effect of AEAL and MEAL on lipid profile

The level of serum total cholesterol was increased in all the diabetic groups on ‘0’ day. Treatment with MEAL, AEAL and glibenclamide significantly (p < 0.01) decreased the elevated total cholesterol level from the first week of treatment onwards and this effect was observed throughout till the end of the study (Table no. 32). Similarly there was also a rise in the level of serum triglycerides with diabetic animals. MEAL and AEAL significantly (p < 0.01) decreased the elevated serum triglycerides after 1 week of initiation of treatment and it were observed throughout the study (Table no. 33).

5.1.3.4 Effect of AEAL and MEAL on body weight

Body weight of STZ induced diabetic rats was found to be significantly (p < 0.01) less compared to normal rats. After 1 week of treatment with MEAL and AEAL, the body weight had significantly (p < 0.01) increased compared to diabetic control. Progress in weight gain
of animals in drug treated group was continued to be observed till the end of the study (Table no. 34).

5.1.3.5 Effect of AEAL and MEAL on biochemical parameters
The efficacy of MEAL and AEAL at the dose of 200 mg/ kg and 400 mg/ kg on serum SGOT, SGPT, ALP, BUN, creatinine and total bilirubin in diabetic rats was evaluated. The above biochemical parameters were significantly (p < 0.01) altered in STZ induced diabetic rats compared to normal control rats. In diabetic rats, administration of both doses of MEAL and AEAL and glibenclamide significantly (p < 0.05, p < 0.01) reduced SGOT, SGPT, ALP, BUN and creatinine and increased the T. bilirubin level (p < 0.01) compared to diabetic control rats (Table no. 35).

5.1.3.6 Effect of MEAL and AEAL on total protein by Biuret method
There was significant decrease in total protein in diabetic rats. Treatment with MEAL and AEAL significantly increase (p < 0.01) the total protein (Table no. 36).

5.1.3.7 Effect of MEAL and AEAL on antioxidant enzymes
The level of antioxidant enzymes viz. catalase, SOD, GPX and GSH have significantly decreased in the diabetic control animals. The treatment with MEAL and AEAL has significantly (p < 0.01) increased the levels of catalase, SOD, GPX and GSH (Table no. 36 and 37).

5.1.3.8 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. This rise in the MDA was significantly (p < 0.01) lowered on treatment with MEAL and AEAL (Table no. 36).

5.1.3.9 Effect of MEAL and AEAL on hepatic glucose – 6 - phosphatase, fructose 1, 6 – bisphosphatase
The level of hepatic glucose-6-phosphatase, fructose 1, 6 – bisphosphatase was significantly increased in the diabetic control animals. The treatment with MEAL and AEAL has significantly (p < 0.01) decreased the level of glucose-6-phosphatase, fructose 1, 6 – bisphosphatase in the liver (Table no. 37).
5.1.3.10 Effect of MEAL and AEAL on hexokinase and 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 hexokinase and liver glycogen content (Table no. 37).

5.1.3.11 Histopathology on MEAL and AEAL
The gross pathological evaluation of brain, eyes, heart, liver, kidneys and pancreas of the rats from the diabetic control and treatment groups revealed no abnormalities and remained similar to those of vehicle control animals. The histopathological examination of hematoxylin and eosin stained sections of brain, eyes, heart, liver, kidneys and pancreas of all the rats from vehicle control group revealed normal histological pattern.
The diabetic control rats administered with 60 mg STZ/ kg b.wt, revealed multifocal hepatocellular hypertrophy with intracytoplasmic microvesicular steatosis, bridging coagulative necrosis of centrilocular hepatocytes characterized by cytoplasmic eosinophilia, karyolysis and mononuclear cell infiltration in the liver, atrophy of 50 – 60 % of islets of langerhans accompanied by marked vacuolar degeneration of islet cells and mild to moderate multifocal mononuclear cell infiltration in the pancreas.
Few islets (10 - 20 %) in the pancreas of diabetic rats treated with the low doses of AEAL (200 mg/ kg b. wt) and MEAL (200mg/ kg b. wt) extracts exhibited degenerative and atrophic changes characterized by β-cell vacuolation and marked reduction in their size, respectively. While, other organs such as brain, eyes, heart and kidneys were found to be histologically normal and remained similar to those of vehicle control group (Fig. 60 to Fig. 87). The histopathological evaluation of brain, eyes, heart, liver, kidneys and pancreas from the diabetic rats treated with higher doses of AEAL at 400 mg/ kg b. wt, MEAL at 400 mg/ kg b. wt and glibenclamide revealed normal histological pattern, respectively and remained similar to those of vehicle control group (Fig. 93, 95, 100 and 102).
These findings suggest that the pathological progression of streptozotocin induced diabetes were found to be suppressed by the treatment of MEAL and AEAL at the doses of
400 mg/ kg b. wt, respectively for 14 days and were comparable to those of glibenclamide treatment.

5.1.4 Discussion

In light of the results, our study indicates that aerial parts of *Aerva lanata* Linn Juss have significant antihyperglycemic activities in streptozotocin (STZ) induced hyperglycaemic rats. They can also improve the condition of diabetes as indicated by parameters like lipid and biochemical parameters. Streptozotocin was known to destroy the β-cells of the pancreas, which causes selective pancreatic islet β-cell cytotoxicity mediated through the release of nitric oxide (NO), methyl cations, methyl radicals, reactive oxygen species (ROS). This results in rapid reduction in pancreatic islet pyridine nucleotide concentration and subsequent β-cell necrosis. The action of STZ on mitochondria generates SOD anions, which leads to diabetic complications (Yadav et al., 2008; Papaccio et al., 2000; Anderson et al., 1974). Based on the above perspectives, in the present study, the oxidative stress has been assessed in rats made diabetic by STZ. Sulfonylureas such as glibenclamide are often used as a standard antidiabetic drug in STZ-induced diabetes to compare the efficacy of variety of antihyperglycemic compounds. It has been involved in stimulating insulin secretion from pancreatic β-cells principally by inhibiting ATP sensitive K - ATP channels in the plasma membrane. In our study, there was a significant elevation in blood glucose level in diabetic control group as compared with normal animals. The MEAL and AEAL treated group exhibited significant reduction of fasting plasma glucose levels as compared to the diabetic control group(Table no. 31). Over production of glucose by means of excessive hepatic glycogenolysis and gluconeogenesis is one of the fundamental basis of hyperglycemia in diabetes mellitus (Szkudelski, 2001). The most commonly observed lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia (Shepherd, 2005)and (Shirwaikar et al., 2005).This might have occurred in the diabetic rats as a result of lack of insulin which activates the lipase enzymes, hydrolyzing the stored TG and releasing large amounts of fatty acids and glycerol in the circulating blood (Shirwaikar et al., 2006) Consequently, the excess of fatty acids in the plasma may promote the hepatic conversion
of fatty acids into phospholipids and cholesterol, the main product of lipid metabolism (Swanson-Flat et al., 1990). The increase level of TG and cholesterol in the blood of diabetic rats may lead to cardiovascular disease. The improvements in the lipid profile in diabetic animals after treatment with MEAL and AEAL could be beneficial in preventing diabetic complications as well as improving lipid metabolism (Cho et al., 2002). The decrease in body weight with diabetes mellitus has been attributed to the gluconeogenesis i.e., catabolism of proteins and fats, which is associated with the characteristic loss of body weight due to increased muscle wasting and loss of tissue proteins (Latner, 1958) and (Shirwaikar et al., 2004). In the present study, diabetic rats treated with MEAL and AEAL showed an increase in body weight as compared to the diabetic control, which may be due to its protective effect in controlling muscle wasting i.e., reversal of gluconeogenesis (Table no. 34). The ability of aerial parts of Aerva lanata Linn Juss in effectively controlling the increase in blood glucose levels in the diabetic group of rats and significantly increasing the body weight may be attributed to its anti hyperglycemic activity. Elevation of serum biomarker enzymes such as SGOT, SGPT and SALP were observed in diabetic rats indicating impaired liver function, which is obviously due to hepatocellular necrosis. Diabetic complications such as increased gluconeogenesis and ketogenesis may be due to elevated transaminase activities (Ghosh and Suryawansi, 2001). Bilirubin is formed from degeneration of haemoglobin by the action of reticuloendothelial systems throughout the body. Increased bilirubin level reflects the depth of jaundice (Pieme et al., 2006; Tedong et al., 2008). Restoration of these biomarker enzymes towards normal level indicates decreased diabetic complications in MEAL and AEAL treated groups. Elevated levels of urea are observed during increased protein breakdown (Mohammadi and Naik, 2008). In the present investigation, the elevated level of serum creatinine and urea levels are considered as obvious indicators for kidney damage and dysfunction. The diabetic hyperglycemia induces elevations of blood levels of creatinine urea which are considered as significant markers of renal dysfunction. In our study, it was observed that the levels of antioxidant enzymes (SOD, CAT, GPx, and GSH) and the lipid peroxidation levels were decreased in
kidney of diabetic rats. The plasma renal markers also showed changes in levels. 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 no. 36 and 37). This might reflect the antioxidant potency, which by reducing blood glucose levels prevented glycation and inactivation of enzymes. The decrease in lipids and urea levels on treatment with MEAL and AEAL suggests that the gluconeogenesis is in control and substantiates that the mechanism of antidiabetic activity may be due to improvisation of glucose utilization, thereby decreasing gluconeogenesis. The mechanism of action of α-glucosidase inhibitors is the inhibition of glucose absorption and hence, does not contribute for decrease in the gluconeogenesis.

Streptozotocin induced diabetic rats showed decrease in the plasma protein content. The reduction in plasma protein was mainly due to progressive protenuria followed by a gradual decline in renal function (Latha and Daisy, 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. Increased oxidative stress is involved in diabetes. 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). 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 no.36). 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 and Pari, 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 no. 36) and may thereby help to control free radicals, as Aerva lanata Linn Juss has been reported to be rich in flavonoids and 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 and Godin, 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
*Aerva lanata* Linn Juss, which may, in turn maintain the antioxidant status in the tissues of diabetic rats. This indicates that the extract can reduce the oxidative stress leading to less degradation of GSH, or have both effects.

The effects of MEAL and AEAL on hexokinase, glucose-6-phosphatase, fructose-1, 6-bisphosphatase and liver glycogen in diabetic rats are shown in Table no.37. Flavanoids, glycosides stimulate the secretion of insulin in β-cells of pancreas (Hii and Howell, 1985). In glucose loaded animals, it is possible that the compound may act by potentiation the pancreatic secretion or increasing glucose uptake. On the basis of above evidence it is possible that the presence of glycosides and tannins are responsible for the activity (Chauhan and Dixit, 2007). In general, increased hepatic glucose production, plus decreased hepatic glycogen synthesis and glycolysis, are the major symptoms in type 2 diabetes that result in hyperglycemia (Jung *et al*., 2004). Hepatic glucokinase is the most sensitive indicator of the glycolytic pathway in diabetes and its increase can increase the utilization of blood glucose for glycogen storage in the liver (Lynedjian *et al*., 1988). In the current study, the effect of *Aerva lanata* in experimental diabetic rats increased hepatic glucokinase. Also, hepatic glycogen reserves are important for whole body glucose homeostasis and are markedly low in the diabetic state (Hornbrook, 1970; Migliorini, 1971). In the current study, the hepatic glycogen concentration was significantly higher in the *Aerva lanata* Linn Juss compared with the control group.

The gluconeogenic enzyme glucose-6-phosphatase is a crucial enzyme of glucose homeostasis because it catalyses the ultimate biochemical reaction of both glycogenolysis and gluconeogenesis (Mithievre *et al*., 1996). These seem to be the consequence of the high glucose-6-phosphatase activities in a diabetic state (Defronzo, 1988; Guignot and Mithieux, 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 and Convery, 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). Gluconeogenic enzyme activation is due to the state of insulin impairment because under normal conditions, insulin functions as a suppressor of gluconeogenic enzymes (Pari and Murugan, 2005).

Glycogen is the primary intracellular storable form of glucose and its levels in various tissues especially skeletal muscle are a direct reflection of insulin activity as insulin promotes intracellular glycogen deposition by stimulating glycogen synthetase and inhibiting glycogen phosphorylase. 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 (Whitton and Hems, 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.

Streptozotocin was known to destroy the β-cells of the pancreas. Histopathology studies showed that there were a reduced number of β-cells in diabetic animals. There was no change in the cells on treatment with MEAL, AEAL or glibenclamide, which suggest that there is no regeneration of β-cells or no insulinogenic property.

The histopathology reveals that the site of action may be extrapancreatic and not the regeneration of β-cells and the decrease in blood glucose may be attributed to the stimulation of glucose uptake by peripheral tissues and decrease in the gluconeogenesis. Hence, the antihyperglycemic effect may be probably brought about by an extrapancreatic mechanism.

Phytochemical studies on *Aerva lanata* Linn Juss revealed that it contains flavanoid glycosides, aervitrin, aervolamine, aervoside and vanillic acid (Vetrichelvan and Jegadeesan, 2002). Beneficial multiple activities like manipulating carbohydrate metabolism by various mechanisms, preventing and restoring the integrity and function of β-cells, releasing insulin activity, improving glucose uptake and utilization, and the antioxidant properties present in
medicinal plants, offer an exciting opportunity to develop them into novel therapeutics. Hence further phytopharmacological studies on the basis of its ethno botanical use can help to explore and establish the bioactive constituents which can be used safely for the treatment of various diseases and disorders in future.

5.1.5 Conclusion

The antihyperglycemic, antihyperlipidemic effects and biochemical parameters of MEAL and AEAL of aerial parts of *Aerva lanata* Linn. Juss mediated through the peripheral mechanisms and the effects may be attributed to the components such as flavonoids, triterpenoids and antioxidant principles present in the MEAL and AEAL. The present findings may pave the way for the bioactivity guided fractionation and the isolation of novel lead compounds in *Aerva lanata* for the antidiabetic activity which will be useful for the design and synthesis of potent antidiabetic and antihyperlipidemic components hence beneficial for the patients. However, further studies are underway to isolate the lead molecule(s) responsible for the activity and also to pinpoint on the mechanism of action of the same.
Table no. 31 Effect of MEAL and AEAL on fasting blood glucose

<table>
<thead>
<tr>
<th>S. no</th>
<th>Treatment</th>
<th>Blood Glucose (mg/ dl)</th>
<th>0 day</th>
<th>1st week</th>
<th>2nd week</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Normal control</td>
<td>90.08 ± 3.21</td>
<td>85.11 ± 4.14</td>
<td>94.52 ± 3.42</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Diabetic control</td>
<td>268.65 ± 5.81</td>
<td>258.23 ± 8.68</td>
<td>265.96 ± 4.95</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Standard 0.5 mg/ kg</td>
<td>141.01 ± 1.9</td>
<td>135.51 ± 1.71**</td>
<td>132.01 ± 0.68**</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>MEAL 200 mg/ kg</td>
<td>263.83 ± 4.72</td>
<td>184.05 ± 2.46**</td>
<td>147.38 ± 0.73**</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>MEAL 400 mg/ kg</td>
<td>259.45 ± 4.51</td>
<td>175.21 ± 2.6**</td>
<td>140.38 ± 0.98**</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>AEAL 200 mg/ kg</td>
<td>263.83 ± 4.62</td>
<td>192.66 ± 1.71**</td>
<td>146.83 ± 0.56**</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>AEAL 400 mg/ kg</td>
<td>253.25 ± 4.54</td>
<td>187.5 ± 1.95**</td>
<td>144.33 ± 1.95**</td>
<td></td>
</tr>
</tbody>
</table>

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. ’0 day’ indicates the initial day in which the treatment commenced.
Table no. 32 Effect of MEAL and AEAL on cholesterol

<table>
<thead>
<tr>
<th>S. no</th>
<th>Treatment</th>
<th>Cholesterol (mg/ dl)</th>
<th>0 day</th>
<th>1\textsuperscript{st} week</th>
<th>2\textsuperscript{nd} week</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal control</td>
<td>67.92 ± 2.68</td>
<td>65.49 ± 1.04</td>
<td>61.55 ± 0.49</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Diabetic control</td>
<td>170.41 ± 0.99</td>
<td>168.37 ± 2.81</td>
<td>162.32 ± 1.22</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Standard 0.5 mg/ kg</td>
<td>106.3 ± 1.55</td>
<td>73.63 ± 0.88**</td>
<td>71.13 ± 0.38**</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>MEAL 200 mg/ kg</td>
<td>165.79 ± 1.54</td>
<td>98.18 ± 0.48**</td>
<td>93.85 ± 0.89**</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>MEAL 400 mg/ kg</td>
<td>163.84 ± 1.65</td>
<td>93.12 ± 0.55**</td>
<td>81.79 ± 0.74**</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>AEAL 200 mg/ kg</td>
<td>167.35 ± 1.54</td>
<td>104.35 ± 0.33**</td>
<td>100.2 ± 0.34**</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>AEAL 400 mg/ kg</td>
<td>165.09 ± 1.65</td>
<td>95.5 ± 0.36**</td>
<td>88.84 ± 0.87**</td>
<td></td>
</tr>
</tbody>
</table>

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. ‘0 day’ indicates the initial day in which the treatment commenced.
Table no. 33 Effect of MEAL and AEAL on triglycerides

<table>
<thead>
<tr>
<th>S. no</th>
<th>Treatment</th>
<th>Triglycerides (mg/ dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0day</td>
</tr>
<tr>
<td>1</td>
<td>Normal control</td>
<td>87.11 ± 2.11</td>
</tr>
<tr>
<td>2</td>
<td>Diabetic control</td>
<td>261.31 ± 7.61</td>
</tr>
<tr>
<td>3</td>
<td>Standard 0.5 mg/ kg</td>
<td>154.01 ± 6.69</td>
</tr>
<tr>
<td>4</td>
<td>MEAL 200 mg/ kg</td>
<td>245.63 ± 2.24</td>
</tr>
<tr>
<td>5</td>
<td>MEAL 400 mg/ kg</td>
<td>238.88 ± 0.32</td>
</tr>
<tr>
<td>6</td>
<td>AEAL 200 mg/ kg</td>
<td>253.83 ± 2.43</td>
</tr>
<tr>
<td>7</td>
<td>AEAL 400 mg/ kg</td>
<td>243.16 ± 2.1</td>
</tr>
</tbody>
</table>

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. ‘0 day’ indicates the initial day in which the treatment commenced.
Table No. 34 Effect of MEAL and AEAL on body weight

<table>
<thead>
<tr>
<th>S. no</th>
<th>Treatment</th>
<th>Body weight (gms)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 day</td>
<td>1st week</td>
<td>2nd week</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Normal control</td>
<td>159.16 ± 6.77</td>
<td>162.66 ± 0.88</td>
<td>161.83 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Diabetic control</td>
<td>161 ± 7.12</td>
<td>133.66 ± 1.3</td>
<td>122.8 ± 2.22</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Standard 0.5 mg/kg</td>
<td>160 ± 6.47</td>
<td>161.66 ± 4.96**</td>
<td>153.5 ± 1.47**</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>MEAL 200 mg/kg</td>
<td>160.16 ± 4.72</td>
<td>152.5 ± 0.34**</td>
<td>141.36 ± 0.47**</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>MEAL 400 mg/kg</td>
<td>161.83 ± 8.39</td>
<td>157.16 ± 4.86**</td>
<td>146.6 ± 0.5**</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>AEAL 200 mg/kg</td>
<td>157.83 ± 8.34</td>
<td>155.16 ± 0.4**</td>
<td>139.4 ± 0.24**</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>AEAL 400 mg/kg</td>
<td>161.33 ± 6.25</td>
<td>156.5 ± 1.31**</td>
<td>144.2 ± 0.37**</td>
<td></td>
</tr>
</tbody>
</table>

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. ‘0 day’ indicates the initial day in which the treatment commenced.
### Table No. 35 Effect of MEAL and AEAL on biochemical parameters

<table>
<thead>
<tr>
<th>S.no</th>
<th>Treatment</th>
<th>SGOT (IU/ dl)</th>
<th>SGPT (IU/ dl)</th>
<th>Creatinine (mg/ dl)</th>
<th>ALP (IU/ dl)</th>
<th>BUN (mg/ dl)</th>
<th>T. Bilirubin (mg/ dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal Control</td>
<td>169.66 ± 17.14</td>
<td>123.17 ± 1.91</td>
<td>1.64 ± 0.04</td>
<td>224.08 ± 13.33</td>
<td>20.63 ± 1.13</td>
<td>0.63 ± 0.03</td>
</tr>
<tr>
<td>2</td>
<td>Diabetic control</td>
<td>297.46 ± 62.17</td>
<td>208.3 ± 37.83</td>
<td>1.85 ± 0.01</td>
<td>290.03 ± 21.97</td>
<td>38.87 ± 0.67</td>
<td>0.4 ± 0.04</td>
</tr>
<tr>
<td>3</td>
<td>Standard</td>
<td>180.33 ± 12.21**</td>
<td>121.43 ± 9.59**</td>
<td>1.69 ± 0.009**</td>
<td>229.35 ± 10.38</td>
<td>21.91 ± 1.81**</td>
<td>0.61 ± 0.03**</td>
</tr>
<tr>
<td>4</td>
<td>MEAL (200 mg/ kg)</td>
<td>189.60 ± 12.01**</td>
<td>122.58 ± 6.41**</td>
<td>1.71 ± 0.02**</td>
<td>231.4 ± 6.79**</td>
<td>28.2 ± 1.26**</td>
<td>0.56 ± 0.02**</td>
</tr>
<tr>
<td>5</td>
<td>MEAL (400 mg/ kg)</td>
<td>183.93 ± 14.03**</td>
<td>111.4 ± 5.98**</td>
<td>1.72 ± 0.01**</td>
<td>228.88 ± 10.5**</td>
<td>22.39 ± 2.52**</td>
<td>0.58 ± 0.03**</td>
</tr>
<tr>
<td>6</td>
<td>AEAL (200 mg/ kg)</td>
<td>204.00 ± 12.1*</td>
<td>128.54 ± 10.33**</td>
<td>1.7 ± 0.02**</td>
<td>233.86 ± 7.15*</td>
<td>30.02 ± 0.52**</td>
<td>0.56 ± 0.02**</td>
</tr>
<tr>
<td>7</td>
<td>AEAL (400 mg/ kg)</td>
<td>196.91 ± 7.89*</td>
<td>135.29 ± 9.65**</td>
<td>1.69 ± 0.01**</td>
<td>232.68 ± 6.56**</td>
<td>28.82 ± 2.22**</td>
<td>0.58 ± 0.03**</td>
</tr>
</tbody>
</table>

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 hyperglycaemic control at p < 0.05. '0 day' indicates the initial day in which the treatment commenced.
Table No. 36 Bio assays of MEAL and AEAL

<table>
<thead>
<tr>
<th>S. No</th>
<th>Treatment</th>
<th>Protein (mg/ml)</th>
<th>Catalase (mcg/mg prtn/ ml)</th>
<th>Superoxide dismutase (unit/mg prtn)</th>
<th>Lipid peroxidation (mcg/ mg prtn/ml)</th>
<th>Glutathione Peroxidase (mcg/mg. prtn/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal Control</td>
<td>9.38 ± 0.51</td>
<td>12.44 ± 0.14</td>
<td>11.97 ± 0.40</td>
<td>10.63 ± 0.21</td>
<td>11.52 ± 0.48</td>
</tr>
<tr>
<td>2</td>
<td>Hyperglycemic</td>
<td>6.63 ± 0.18</td>
<td>5.56 ± 0.19</td>
<td>6.52 ± 0.16</td>
<td>12.94 ± 0.37</td>
<td>7.89 ± 0.19</td>
</tr>
<tr>
<td>3</td>
<td>Standard</td>
<td>8.81 ± 0.28</td>
<td>10.6 ± 0.22 **</td>
<td>10.24 ± 0.50**</td>
<td>10.01 ± 0.28**</td>
<td>11.61 ± 0.89**</td>
</tr>
<tr>
<td>4</td>
<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>9.25 ± 0.06**</td>
<td>10.73 ± 0.36**</td>
</tr>
<tr>
<td>5</td>
<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>9.92 ± 0.12**</td>
<td>10.99 ± 0.31**</td>
</tr>
<tr>
<td>6</td>
<td>AEAL (200mg/ kg)</td>
<td>7.92 ± 0.08**</td>
<td>8.55 ± 0.23 **</td>
<td>8.31 ± 0.40**</td>
<td>8.87 ± 0.18**</td>
<td>10.34 ± 0.42**</td>
</tr>
<tr>
<td>7</td>
<td>AEAL (400mg/ kg)</td>
<td>8.04 ± 0.02**</td>
<td>9.98 ± 0.16**</td>
<td>8.59 ± 0.33**</td>
<td>9.07 ± 0.21**</td>
<td>10.38 ± 0.22**</td>
</tr>
</tbody>
</table>

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.
<table>
<thead>
<tr>
<th>S. no</th>
<th>Treatment</th>
<th>Reduced Glutathione (mg/ dl)</th>
<th>Glucose 6-Phosphatase (mcg/ mg. prtn/ ml)</th>
<th>Fructose 1,6 biphosphatase (mcg/ mg. prtn/ ml)</th>
<th>Hexokinase (mcg/ mg. prtn/ ml)</th>
<th>Liver Glycogen (mg/ dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<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>
<td>689.34 ± 24.93</td>
</tr>
<tr>
<td>2</td>
<td>Positive 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>
<td>527.53 ± 7.08</td>
</tr>
<tr>
<td>3</td>
<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>
<td>681.72 ± 33.85**</td>
</tr>
<tr>
<td>4</td>
<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>
<td>674.15 ± 32.66**</td>
</tr>
<tr>
<td>5</td>
<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>
<td>679.64 ± 22.22**</td>
</tr>
<tr>
<td>6</td>
<td>AEAL (200 mg/ kg)</td>
<td>54.64 ± 0.84**</td>
<td>607.56 ± 4.65**</td>
<td>754.03 ± 4.03**</td>
<td>759.94 ± 19.94**</td>
<td>661.29 ± 33.66**</td>
</tr>
<tr>
<td>7</td>
<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>
<td>665.18 ± 32.80**</td>
</tr>
</tbody>
</table>

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 hyperglycaemic control at p < 0.05.
Fig. 51 Effect of MEAL and AEAL on blood glucose

Fig. 52 Effect of MEAL and AEAL on cholesterol

Fig. 53 Effect of MEAL and AEAL on triglycerides
Fig. 54 Effect of MEAL and AEAL on body weight

Fig. 55 Effect of MEAL and AEAL on SGOT, SGPT and ALP

Fig. 56 Effect of MEAL and AEAL on creatinine and T. bilirubin
Fig. 57 Effect of MEAL and AEAL on BUN and reduced glutathione

I – BUN, II – Reduced glutathione

Fig. 58 Bio assays of MEAL and AEAL

I-Protein; II – Catalase ; III – Superoxide dismutase ; IV – Lipid peroxidation; V – Glutathione peroxidase

Fig. 59 Bio assays of MEAL and AEAL

I – Glucose 6- phosphatase ;II - Fructose 1,6 biphosphatase ; III – Hexokinase ; IV – Liver glycogen
Histopathology of brain

Fig. 60
Normal control brain cortex

Fig. 61
STZ brain cortex

Fig. 62
Std. brain cortex

Fig. 63
MEAL (200 mg/kg)

Fig. 64
MEAL (400 mg/kg)

Fig. 65
AEAL (200 mg/kg)

Fig. 66
AEAL (400 mg/kg)
Histopathology of eye

Fig. 67
Normal control eye cornea

Fig. 68
STZ eye cornea

Fig. 69
STD eye cornea

Fig. 70
MEAL (200 mg/ kg)

Fig. 71
MEAL (400 mg/ kg)

Fig. 72
AEAL (200 mg/ kg)

Fig. 73
AEAL (400 mg/ kg)
Histopathology of heart

Fig. 74
Normal heart myocardium

Fig. 75
STZ heart myocardium

Fig. 76
STD heart myocardium

Fig. 77
MEAL (200 mg/kg)

Fig. 78
MEAL (400 mg/kg)

Fig. 79
AEAL (200 mg/kg)

Fig. 80
AEAL (400 mg/kg)
**Histopathology of kidney**

Fig. 81  Fig. 82  Fig. 83
Kidney normal cortex  STZ  STD

Fig. 84  Fig. 85  Fig. 86
MEAL (200 mg/ kg)  MEAL (400 mg/ kg)  AEAL (200 mg/ kg)

Fig. 87
AEAL (400 mg/ kg)
Histopathology of liver

Fig. 88
Normal control

Fig. 89
Liver STZ Liver

Fig. 90
STZ liver

Fig. 91
STD

Fig. 92
MEAL (200 mg/ kg)

Fig. 93
MEAL (400 mg/ kg)

Fig. 94
AEAL (200 mg/ kg)

Fig. 95
AEAL (400 mg/ kg)
Histopathology of pancreas

Fig. 96  Fig. 97  Fig. 98
Normal control  STZ Pancreas  STD

Fig. 99  Fig. 100  Fig. 101
MEAL (200 mg/ kg)  MEAL (400 mg/ kg)  AEAL (200 mg/ kg)

Fig. 102
AEAL (400 mg/ kg)
Fig. 89 and Fig. 90: The diabetic control rats administered with 60 mg STZ/ kg b.wt revealed multifocal hepatocellular hypertrophy with intracytoplasmic microvesicular steatosis, bridging coagulative necrosis of centrilobular hepatocytes characterized by cytoplasmic eosinophilia, karyolysis and mononuclear cell infiltration in the liver.

Fig. 91: Glibenclamide liver periportal hypertropic hepatocytes

Fig. 96: Islets of Langerhans and acinar cells

Fig. 97: Atrophy of 50 – 60 % of islets of langerhans accompanied by marked vacuolar degeneration of islet cells and mild to moderate multifocal mononuclear cell infiltration in the pancreas.

Fig. 99 and 101: Few islets (10 - 20 %) in the pancreas of diabetic rats treated with the low doses of AEAL (200 mg/ kg b. wt) and MEAL (200 mg/ kg b. wt) extracts exhibited degenerative and atrophic changes characterized by β-cell vacuolation and marked reduction in their size, respectively.

Fig. 60 to Fig. 87: While, other organs such as brain, eyes, heart and kidneys were found to be histologically normal and remained similar to those of vehicle control group.

Fig. 93, 95, 100 and 102: The histopathological evaluation of liver and pancreas from the diabetic rats treated with higher doses of AEAL at 400 mg/ kg b. wt, MEAL at 400 mg/ kg b. wt and glibenclamide revealed normal histological pattern, respectively and remained similar to those of vehicle control group.
References


Gancedo JM, Gancedo C. Fructose 1,6 diphosphatase, phosphor-fructokinase and glucose-6-phosphate dehydrogenase from fermenting and non fermenting yeasts, *Arch Microbiol* 1971; **76**: 132 – 138.


Lee AY, Chung SK, Chung SS. Demonstration that polyol accumulation is responsible for diabetic cataract by the use of transgenic mice expressing the aldose reductase gene in the lens, *Proc. Natl Acad. Sci. USA* 1995; **92**: 2780 – 2784.


Migliorini RH. Early changes in the levels of liver glycolytic enzymes after total pancreatectomy in the rat, *Biochemica Biophysica Acta* 1971; **244**: 125 – 128.


Temelkova-Kurktschiev TS. Post challenge plasma glucose and glycemic spikes are more strongly associated with atherosclerosis than fasting glucose or HbA1c levels, *Diabetes Care* 2000;12: 1830 – 1834.


5.2 Comparative evaluation of anticancer activity against Dalton’s Ascitic Lymphoma in Swiss albino mice

5.2.1. Introduction

Cancer is a major public health burden in both developed and developing countries. Plant derived agents are being used for the treatment of cancer. Cancer is expected to claim 9 million deaths worldwide by the year 2015. A major problem in the use of chemo preventing agent in cancer treatment is the potential toxicity of these drug to normal cells. Although there has been increasing sophistication of current therapeutic strategies, 40% of patients are still likely to die from the disease. Novel potent anticancer compounds are needed to address this growing issue of cancer. Despite skeptical connotations, traditional medicine has aroused renewed interest as worldwide efforts continue the search for novel compounds that exhibit potent and selective anticancer properties.

Oncology is the study of tumors or neoplasm. Cancer is the common term for all malignant tumors. The term tumor was originally applied to the swelling caused by inflammation. Although the ancient origin of this term is somewhat uncertain, it probably derives from the Latin word crab, cancer presumably because a cancer “adheres to any part that it seizes upon in an obstinate manner like the crab” (Vinay kumar et al., 2006).

5.2.1.1 Nomenclature

The nomenclature of tumor is, however based on the parenchymal component.

a) Benign Tumor

In general, benign tumors are designated by attaching the suffix-oma to the cell of origine.g. Tumor arising from fibroblastic cell is called a fibroma. In contrast, nomenclature of benign epithelial tumors is more complex. They are variously classified; some based on the cells of origin, others on microscopic architecture, and still others on their macroscopic patterns. e.g. Adenoma is the term applied to a benign epithelial neoplasm that forms glandular patterns as well as tumors derived from gland renal tubular cells growing in the form of numerous tightly clustered small glands would be termed an adenoma.
b) Malignant tumors

Malignant tumors arising in mesenchymal tissue are usually called sarcomas because they have little connective tissue stroma and so are fleshy.

Eg. Fibrosarcoma, liposarcoma, malignant neoplasms of epithelial cell origin, derived from any of the three germ layers, are called carcinomas.

5.2.1.2 Rate of Growth

The rate of growth of a tumor is determined by three main factors

- The doubling time of tumor cells
- The fraction of tumor cells that are in the replicative pool
- The rate at which cells are shed and lost in the growing lesion.

In reality, total cell cycle time for many tumors is equal to or longer than that of corresponding normal cells. Thus, it can be safely concluded that growth of tumor is not commonly associated with a shortening of cell cycle time.

Epidemiology

Because cancer is a disorder of cell growth and behaviour, its ultimate cause has to be defined at the cellular and subcellular levels. Major cause of cancer can be obtained by epidemiologic studies that relate particular environmental, hereditary and cultural influence to the occurrence of malignant neoplasm. In addition, certain diseases are associated with an increased risk of developing cancer.
5.2.1.3 Cancer incidence (Jemal et al., 2005)

Table no. 38

<table>
<thead>
<tr>
<th>Male</th>
<th>%</th>
<th>Female</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melanoma of skin</td>
<td>4</td>
<td>Melanoma of skin</td>
<td>3</td>
</tr>
<tr>
<td>Oropharynx</td>
<td>3</td>
<td>Lung</td>
<td>12</td>
</tr>
<tr>
<td>Pancreas</td>
<td>2</td>
<td>Thyroid</td>
<td>3</td>
</tr>
<tr>
<td>Lungs</td>
<td>14</td>
<td>Breast</td>
<td>32</td>
</tr>
<tr>
<td>Colon and rectum</td>
<td>11</td>
<td>Colon and rectum</td>
<td>11</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>6</td>
<td>Urinary bladder</td>
<td>2</td>
</tr>
<tr>
<td>Prostate</td>
<td>33</td>
<td>Ovary</td>
<td>4</td>
</tr>
<tr>
<td>Leukemia</td>
<td>3</td>
<td>Uterus</td>
<td>6</td>
</tr>
<tr>
<td>Non Hodgkin Lymphoma</td>
<td>4</td>
<td>Non Hodgkin Lymphoma</td>
<td>4</td>
</tr>
<tr>
<td>All others</td>
<td>17</td>
<td>All others</td>
<td>20</td>
</tr>
</tbody>
</table>

5.2.1.4 Geographic and environmental factors

In general it is believed that most of these geographic differences are the consequence of environmental influences.

e.g. The death rate for stomach carcinoma in both men and women is 7 to 8 times higher in Japan than in the US. In contrast the death rate from carcinoma of the lung is slightly more than twice as great in the US as in Japan. The ultraviolet (UV) rays, asbestos, vinyl chloride and 2- naphthylamine serve as examples of occupational hazards.

- Cigarette smoking has been called the single most important environmental factor contributing to premature death in the U.S.
- Alcohol and tobacco cause cancer in the upper aero digestive tract.
- Age: Most carcinomas occur in the later years of life (> 55 years). Cancer is the main cause of death among women aged 40 to 79 and among men aged 60 to 79.
Genetic: Genes that are casually associated with cancers that have a strong hereditary component are generally also involved in the much more common sporadic forms of the same tumor.

Genetic predisposition to cancer can be divided into three categories.

a) Autosomal dominant inherited cancer syndrome

Inherited cancer syndromes include several well defined cancers in which inheritance of single mutant gene generally increases the risk of developing a tumor.

b) Defective DNA repair syndromes

Diseases which occur rarely are Xeroderma pigmentosum, ataxiatelangectasia and bloom syndrome characterized by genetic instability resulting from defect in DNA repair genes.

c) Familial cancers:

Besides the inherited syndromes of cancer susceptibility, cancer may occur at higher frequency in certain families without a clearly defined pattern of transmission.

e.g. Carcinomas of colon, breast, brain and ovary

Non genetic factors

Even in tumors with a well defined inherited component, the risk of developing the tumor can be greatly influenced by nongenetic factors.

Eg: Breast cancer risk in female carries of BRCA-1 or BRCA-2 mutations is almost three fold higher for women born after 1940, compared to the risk for women born before that year (King et al., 2003).

5.2.1.5 Non hereditary conditions

i) Chronic inflammation and cancer

Increased risk of cancer development in patient affected by a variety of chronic inflammatory disease of the gastrointestinal tract; these include ulcerative colitis, crohn disease, H.pyroli gastritis. The precise mechanism that link inflammation and cancer development have not been established.
ii) Precancerous condition

Certain non neoplastic disorders- the chronic atrophic gastritis of pernicious anaemia, solar ketosis of the skin, chronic ulcerative colitis and leukoplakia of the oral cavity, vulva have such a well defined association with cancer that they have termed precancerous conditions.

iii) Tumor markers (Dan, 2005)

Tumor markers may be useful in patient management in certain tumors. Some tumors produce or elicit the production of markers that can be measured in the serum or urine and in a particular patient; rising and falling levels of the markers are usually associated with increasing or decreasing tumor burden respectively. Tumor markers are not in themselves specific enough to permit a diagnosis of malignancy to be made, but once a malignancy has been diagnosed and shown to be associated with elevated level of a tumor marker, the marker can be used to assess response to treatment. Tumor marker also appears in non-neoplastic condition such as hepatitis, pancreatitis IBD, smoking and ulcerative colitis.

5.2.1.6 Evaluation of carcinogenic effects (Mikheev, 1985)

Evidence of carcinogenicity in humans usually comes from

a) Case reports of individual cancer patients who were exposed to a particular chemical or industrial process.

b) Epidemiological studies in which the incidence of cancer in a human population exposed to a particular factor was higher than in the control group not exposed to the suspected carcinogenic agent.

c) Epidemiological studies described above, the case control study is the most appropriate for assessing the carcinogenicity of occupational factors.

In the absence of sufficient evidence from epidemiological studies alone, evaluations of the carcinogenic risk to human could be based on the combined consideration of epidemiological and experimental evidences. The structure of chemical compounds in relation to the structure of known carcinogens should be taken into account. Exposure to many potential carcinogens is obviously greater in the work place than elsewhere, making if
relatively easier to identify the adverse effect of such agents in an epidemiological study of occupational risks, provided than an exposure oriented recording system is employed.

In general, although a single study may be indicative of a cause effect relationship, confidence in ferring a causal association is increased when several independent studies are concordant in showing the association, when the association is string when a dose response relationship occurs, or when a reduction in exposure is followed by a reduction in the incidence of cancer.

5.2.1.7 Natural products in cancer treatment

The World Health Organization estimates that approximately 80% of the world’s inhabitants rely on traditional medicine for their primary health care (Farnsworth et al., 1985). Traditional medicine now poses as an invaluable ethnopharmacological approach to the non-random selection of bioactive plants, on the basis of their folkloric medicinal usage. Plants that are used as traditional medicine represent a relevant pool for selecting plant candidates that may have anticancer properties. Plants are considered as valuable sources of bioactive compounds and have been used in almost all cultures and communities for thousands of years. Anticancer plants in its most general sense are cancer preventive plants and cancer healing plants.

Plants have a long history of use in the treatment of cancer. Hartwell, in his review of plants used against cancer, lists more than 3000 plant species that have reportedly been used in the treatment of cancer. It is significant that over 60% of currently used anticancer agents are derived in one way or another from natural sources, including plants, marine organisms and micro-organisms. Indeed, molecules derived from natural sources (so called natural products), including plants, marine organisms and micro-organisms have played and continue to play, a dominant role in the discovery of leads for the development of conventional drugs for the treatment of most human diseases. The search for anti-cancer agents from plant sources started in earnest in the 1950s with the discovery and development of the vinca alkaloids, vinblastine and vincristine, and the isolation of the cytotoxic podophyllotoxins. These discoveries prompted the United States National Cancer
Institute (NCI) to initiate an extensive plant collection program in 1960. This led to the discovery of many novel chemotypes showing a range of cytotoxic activities, including the taxanes and camptothecins (Cragg and Newmann, 2005).

Chemotherapy is an effective treatment against cancers either singly or in combination with surgery and/ or radiotherapy (Black and Livingston, 1990). However, therapeutic efficacies of most of them are limited due to the development of various side effects in the host and / or the acquired drug resistance by the cancer cells (Kartalou and Essigmann, 2001). In an attempt to abate these side effects and better remedy for cancer, many plant derivatives have been used with varying success (Roja and Rao, 2007). More than 50 % of all modern drugs in clinical use are of natural product origin (Huang Paul et al., 1992).

Cancer is one of the ailments which cannot be completely subdued by chemotherapy. The chemotherapeutic agents though effective against various types of tumour are not totally free from side effects. This fostered our attempts to evaluate some plant products against cancer, as they are less likely to cause serious side effects. Many Indian spices (Unnikrishnan and Kuttan, 1990) and plants (Babu et al., 1995) are quoted to be useful in different types of cancer. One such plant is *Aerva lanata Linn* Juss belonging to the family Solanaceae.

India is a rich source of medicinal plants and a number of plant extracts have been used in various systems of medicines such as ayurveda, sidda, unani, etc. to cure various diseases. Only a few of them have been scientifically explored. Plant derived natural products such as flavonoids, terpenes, alkaloids etc have received considerable attention in recent years due to their diverse pharmacological properties including cytotoxic and cancer chemopreventive effects (Roja and Heble, 1994). Within the scientific community, interest in natural compounds is increasing now a day, which is fuelled partly by well-documented limits and adverse effects of current chemotherapy drugs, as well as the ongoing search for better ways to fight the disease. Scientists are now developing newer drugs by using the natural basic skeleton of an isolated component that targets the unique makeup mechanism of cancer cells. A number of natural products have been studied still now for anti cancer activity on various experimental models.
Hence, a major portion of the current pharmacological research is involved with the anticancer drug design customized to fit new molecular targets (Xia et al., 2004). Due to the enormous propensity of plants, which synthesize a variety of structurally diverse bioactive compounds, the plant kingdom is a potential source of chemical constituents with antitumor and cytotoxic activities. Traditionally various plants have long been used in the treatment of cancer (Kintzios, 2006; Indap et al., 2006; Bufalo et al., 2009). A number of Indian medicinal plants have been used for their anticancer activity in the traditional system of medicine, but not all of them have been reported on scientifically (Rangari, 2004). The objective of the present investigation was to study the effect of methanol and aqueous extracts of aerial parts of *Aerva lanata* Linn Juss against intraperitoneally injected Dalton’s ascitic Lymphoma.

**5.2.2 MATERIALS AND METHODS**

**5.2.2.1 Chemicals and reagents**

5- Fluoro uracil, other chemicals and reagents used for the study were of analytical grade and procured from approved organizations.

**5.2.2.2 Evaluation of anticancer activity** (Christina et al., 2004).

a) **Selection, grouping and acclimatization of Experimental animals**

Healthy Swiss albino mice 25 - 30 gms of either sex were obtained from the standard animal house, Madurai, Tamil Nadu, India. The animals were housed in micro nylon boxes in a control environment (temp 25 ± 2°C) and relative humidity of 45 % to 55 % under 12 hrs light / dark cycles with standard laboratory diet and water *ad libitum*. The experimental protocol was approved by the Institutional animal ethics committee (Reg no 661/02/c/CPCSEA), Approval No RR/ 129/ Ph.D/ III/ 07. CPCSEA guidelines were adhered during the maintenance and experiment.

b) **Acute oral toxicity studies**

Healthy adult albino mice of either sex were subjected to acute toxicity studies as per guidelines suggested by the Organization for Economic Cooperation and Development (OECD 2001). Healthy young adult albino mice of commonly used laboratory strains were employed. Each animal at the commencement of its dosing was between 8 and 12 weeks.
old and its weight was in an interval within ± 20 % of the mean weight of any previously
dosed animals. The temperature in the experimental room was 22°C (± 3°C). For feeding,
conventional laboratory diets was used with an unlimited supply of drinking water. The
animals are randomly selected marked enabling identification and kept in their cage for at
least 5 days prior to dosing to allow for acclimatization to the laboratory conditions. The test
substance was administered in a single dose by gavage using a stomach tube or a suitable
intubation canula. The animals were observed continuously for first 2 h for any gross change
in behavioural, neurological and autonomic profiles or any other symptoms of toxicity and
mortality if any, and intermittently for the next 6 h and then again after 24 h, 48 h and 72 h
for any lethality or death. One-tenth and one-fifth of the maximum safe dose of the extract
tested for acute toxicity were selected for the in vivo experiment (Ghosh, 1984).

c) Induction of Cancer using DLA cells
Dalton’s Lymphoma Ascites (DLA) cells were obtained through courtesy of Amala Cancer
Research Centre, Thrissur, Kerala, India. The cells were maintained in vivo in Swiss albino
mice by intra-peritoneal transplantation. While transforming the tumour cells to the grouped
animal the DLA cells were aspirated from peritoneal cavity of the mice using saline. The cell
count was done and further dilutions were made, so that total cells should be 1 x 10^6 cells/
ml/ mouse. This volume was given intraperitoneally and the tumor was allowed to grow in
the mice for minimum of seven days before starting the study (Gothoskar et al., 1971).

d) Treatment protocol
Swiss albino mice were divided into 5 groups of six each (Babu et al, 2002). The animals
belonging to groups 2–5 were injected with DLA cells (1 x 10^6 cells/ ml/ mouse) intraperitoneally, while the remaining group I were served as the normal control group. After
the inoculation, the groups were treated as given below. Group I- Normal control and Group
II- tumour control, both received normal diet and water. Group III- Positive control, treated
with 5– Fluorouracil 20 mg/ kg body weight intraperitoneally. Group IV- treated with MEALat
a dose of 200 mg/ kg body weight, Group V- treated with AEAL at a dose of 200 mg/ kg body
weight.
e) Collection of blood

For the study against DAL cell lines, the MEAL and AEAL dissolved with 2 ml of sterile water administered once daily through orally for 14 days. After the last dose and 24 hr fasting all mice from all the groups were sacrificed; the blood was withdrawn from each mouse by retro orbital plexus method and the following parameters were evaluated.

1) Haematological parameters

The effect of MEAL and AEAL on haematological parameters was studied in the mice of all groups. Blood was collected from the all mice in the groups by puncturing retro-orbital plexus and counted for RBC, WBC, haemoglobin and platelets and packed cell volume (PCV). Haematological tests were carried out in Cobas Micros OT 18 from Roche.

2) Serum enzyme and Lipid profile

The effect of MEAL and AEAL on serum enzyme and lipid profile like total cholesterol, triglycerides, AST, ALT, ALP was evaluated. All biochemical investigations were done by using Cobas Mira Plus-S auto analyzer from Roche Switzerland. Hi-tech instruments Max Mat auto analyzer was used for all biochemistry investigation in blood sample.

3) Other parameters

i. Body weight

All the mice were weighed, from the day 1st to 15th day of the study. Average increase in body weight on the 15th day was determined (Spiridon, 2006).

ii. Percentage increase in life span (ILS)

Recording the mortality monitored the effect of Aerva lanata on tumour growth and percentage increase in life span (ILS %) were calculated (Sur and Ganguly, 1994). ILS (%) = \[\frac{\text{Mean survival of treated group}}{\text{Mean survival of control group}} - 1\] x100. An enhancement of life span by 25% or more was considered as effective antitumor response (Mazumder et al., 1997; Gupta et al., 2000).

iii. Cancer cell count (Mary et al., 1994)

The fluid (0.1 ml) from the peritoneal cavity of each mouse was withdrawn by sterile syringe and diluted with 0.8 ml of ice cold normal saline or sterile Phosphate buffer solution (PBS)
and 0.1 ml of tryphan blue (0.1 mg/ml) and total number of the living cells were counted using haemocytometer.

- All biochemical investigations were done by using COBAS MIRA PLUS-S from Roche Switzerland
- Haematological tests are carried out in COBAS MICROS OT 18 from Roche.
- Newly added Hi-Tech instrument MAX MAT used for an auto analyser for all biochemistry investigations in blood sample.

f) **Histopathology studies**

On day 14th after the completion of experiment, the animals were fasted overnight. All animals from each group were sacrificed by cervical dislocation under general anaesthesia. The thigh region and liver from all the rats were examined for gross pathological findings. The organs were collected and fixed in 10% neutral buffered formalin for 48 hours, processed for paraffin embedment, sectioned and stained with hematoxylin and 1% eosin and observed under microscope for histopathological evaluation. The alteration and changes in histology of the above mentioned tissues were shown in plates.

g) **Statistical analysis**

The experimental results were expressed as mean ± SEM of six animals. Analysis of variance was performed by one way anova followed by Newman – Keul’s multiple range tests. Probability values less than (p < 0.01) were considered significant.

**5.2.3 Results**

**5.2.3.1 Acute toxicity studies**

In the acute toxicity studies, the dose level to be used as the starting dose was selected as 300 mg/kg body weight. The starting dose level should be that which is most likely to produce mortality in some of the dosed animals. The dose was administered to three animals. After 24 hrs none of the animals died. Hence the next dose at the level of 300 mg/kg was attempted. Following this dose also none of the animals died. Hence the final dose
tried was 2000 mg/kg out of 3 animals 2 died within 24 hrs of administration. Hence the LD_{50} was concluded to be 2000 mg/kg. So the ED_{50} value was calculated 200 mg/kg.

### 5.2.3.2 Effect of MEAL and AEAL on haematological parameters

Regarding the haematological parameters, cancer control mice showed reduced RBC count but increase in WBC count than normal group. The treatment with MEAL, AEAL also raised the RBC count significantly to $2.72 \pm 0.24$ million/ cu. mm, $2.86 \pm 0.21$ million/ cu. mm respectively. Similarly both extracts restored the WBC value to $14.2 \pm 0.30$ cells/ ml $\times 10^3$, $14.6 \pm 0.32$ cells/ ml$\times 10^3$ respectively. Hb content in cancer control mice decreased significantly when compared with normal group. But, the MEAL and AEAL increased Hb content to $7.45 \pm 0.20$ gm/ dl, $7.50 \pm 0.40$ gm/ dl. MEAL and AEAL restored the normal platelet count in tumor induced extract treated mice. Haematological studies exhibited an increase in WBC count in cancer control and this was reduced after treatment with significantly when compared with normal group. But, the MEAL and AEAL decrease PCV content to $26.20 \pm 0.25$ and $26.41 \pm 0.30$. All the values were found to be significantly different from cancer control group at $p < 0.01$ (Table no. 40).

### 5.2.3.3 Effect of MEAL and AEAL on Biochemical Parameters

The inoculation of DLA cells caused significant increase in the level of total cholesterol, triglyceride, Aspartate transaminase (AST), Alanine transaminase (ALT), Alkaline phosphatase (ALP) in serum when compared to normal group. The treatment with MEAL, AEAL at a dose of 200mg/kg reduced the total cholesterol to $131.19 \pm 1.03$ and $130.21 \pm 1.11$ and triglycerides to $203.70 \pm 2.32$ and $197.40 \pm 2.45$. MEAL and AEAL also reduced AST to $79.73 \pm 0.30$, $78.45 \pm 0.22$, ALT to $51.50 \pm 0.54$ and $50.50 \pm 0.45$ and ALP to $224.88 \pm 2.27$ and $226.70 \pm 2.35$ as compare to that of cancer control (Table no. 41). All the values were found to be significantly different from cancer control group at $p < 0.01$. The treatment with standard 5 FU also gave similar significant results at $p < 0.001$ (Fig. 105).

### 5.2.3.4 Other parameters

The effect of MEAL and AEAL on the survival of DLA tumour bearing mice is shown in (Table no. 39). The percentage ILS of the cancer control group was 44 %, whereas it was 88 %, 72
% and 76 % for the groups treated with 5 FU (20 mg/ kg), MEAL and AEAL (200 mg/ kg) respectively (p < 0.01). The effect MEAL and AEAL on the average increase in body weight (Table no. 39). The average weight gain of tumor bearing mice was 8.56 ± 0.20 gm, whereas it was 2.24 ± 0.10 gm, 7.20 ± 0.22 and 7.78 ± 0.28 gm for the groups treated with 5 FU (20 mg/ kg), MEAL and AEAL (200 mg/ kg) respectively (p < 0.01). The intraperitoneal inoculation of DAL cells in the mice produces increased proliferation of cells. MEAL reduced the cancer cell count to 2.10 ± 0.18 × 10^6 cells in the treated mice. Similarly AEAL reduced the cancer cell count to 1.96 ± 0.12 × 10^6 cells in the cancer treated mice (Table no. 39). Extracts treatment reduces the tumor weight and hence increased the life span of cancer induced mice.

5.2.3.5 Histopathological results

1) Fig. 111 Section shows skeletal muscle bundle with tumor showing sheets of polygonal to pleomorphic cells with vesicular nuclei. Area of necrosis seen.

2) Fig. 112 Section shows skeletal muscle bundle and tumor showing small areas of necrosis (30 % of tumor shows necrosis.)

3) Fig. 113 Section shows skeletal muscle bundle and tumor showing small areas of necrosis (30 % of tumor shows necrosis)

4) Fig. 114 Section shows Structure of Liver with sheets of hepatocytes separated by sinusoids cartial vein and portal tract appear normal.

5) Fig. 115 Section shows structure of liver with central vein and radiating column of hepatocytes.

6) Fig. 116 Section shows Structure of Liver with sheets of hepatocytes separated by sinusoids cartial vein and portal tract appear normal.

7) Fig. 117 and 118 (A and B) Section shows structure of liver with sheets of hepatocytes separated by sinusoids cartial vein and portal tract appear normal.

8) Fig. 119 and 120 (A and B) Section shows structure of liver with sheets of hepatocytes separated by sinusoids cartial vein and portal tract appear normal.
5.2.4 Discussion

Cancer is a group of more than 100 different diseases characterized by uncontrolled cellular growth, local tissue invasion and distant metastases (Chabner and Collins, 1990) and the free radicals have been implicated in carcinogenesis (Player, 1982). Supportive to this, many plant extracts containing antioxidant principles have been reported to possess antitumour activity (Ruby et al., 1995). Hence plants containing flavonoid glycosides, etc., are constantly being screened for antitumour activity. Some of the active principles present in this plant are reported to be antioxidant. Hence this plant was chosen to study the antitumour activity against DAL. Lymphoma is defined as malignant tumors of lymphoreticular origin i.e. from lymphocytes and histiocytes and their precursor cells. Intraperitoneal inoculation of DAL cells in the mice produced an enormous increase in the cancer cell count which indicated that there is progression of cancer in the animals. For a similar observation, in this study a cancer control group was used. The increased cell count after 10 days confirmed the proliferation of cells in this group. A decrease in cancer cell count as a confirmatory evidence for protection against DAL has been reported (Rajkapoor et al., 2004). In this study also a similar decrease was observed following the administration of the extracts. The reliable criteria for judging the value of anti-cancer drug is the prolongation of life span of animal and decrease of tumor volume and viable tumor cell count and increase of non-viable tumor cell count (Senthilkumar et al., 2008). In this study also an increase in life span was observed with MEAL and AEAL. The DAL bearing mice administered with MEAL and AEAL at 200 mg/kg showed significant increase in average life span compared to the animals of tumor control group. The present results are in accordance with previous report of cyclophosphamide alone and in combination with ascorbic acid against murine ascites Dalton’s lymphoma in mice (Nicol and Prasad, 2006). In DLA tumor bearing mice, a regular rapid increase in ascitic tumor cell volume was observed (Gupta et al., 2004). However the percentage increase in body weight, packed cell volume and number of viable tumor cells were found to be significantly less in MEAL and AEAL treated mice than the tumor control animals, indicating the anti-cancer nature of extracts of Aerva lanata. The Total WBC count
was found to be increased in cancer control, might be a defensive mechanism against cancer cells, whereas RBC, platelets and Hb content decreased in cancer control when compared to normal control (Badami et al., 2003). The anemia encountered in tumor bearing mice is mainly due to reduction in RBC or hemoglobin percentage and this may occur either due to iron deficiency or due to haemolytic or myelopathic conditions (Fenninger et al., 1954). Treatment with MEAL and AEAL brought back the hemoglobin content, RBC and WBC cell count near to normal values. This indicates that Aerva lanata possesses protective action on the heamopoietic system. Abnormal blood lipid profile has been associated with cancer. In Hodgkin lymphoma, high cholesterol level and low triglyceride level has been reported (Raj Kapoor et al., 2002). Abnormal liver function is observed in patients with Hodgkin lymphoma, so that these liver enzyme levels markedly increased in tumor bearing mice. ALT is an enzyme mainly derived from the liver, bones and in lesser amount from intestine, placenta, kidneys and leucocytes. An increase in ALP levels in the serum is frequently associated with the variety of disease. ALP comprises a group of enzyme that catalyzes the hydrolysis of phosphate esters is an alkaline environment, generating an organic radical and inorganic phosphate (Jacquelin et al., 1998; Reichling et al., 1988). Markedly elevated serum ALP, hyper alkaline phosphatasemia, is seen predominantly with more specific disorder; including malignant biliary cirrhosis, hepatic-lymphoma and sarcoidosis. The significantly elevated level of total cholesterol, triglycerides, AST, ALT and ALP in serum of tumor inoculated animal indicated liver damage and loss of functional integrity of cell membrane. The significant reversal of these changes towards the normal by MEAL and AEAL treatment indicates a protective effect on liver functions. In the present study, the biochemical examination of DAL inoculated animal showed marked changes indicating the toxic effect of tumor. The normalization of these effects observed in the serum treated with MEAL and AEAL supported the potent anti-tumor activity of this plants.

The phytochemical screening of plant extracts showed the presence of alkaloids, tannin like phenolic compounds, flavanoids, steroids and terpenoids. The presence of alkaloids, tannin like phenolic compounds, steroids in MEAL and AEAL may contribute the anticancer activity.
of this traditional herb (Vetrichelvan and Jegadeesan, 2002). Flavonoids have been shown to possess anti-mutagenic (Brown, 1980) and antimalignant effect (Hirano et al., 1989). Moreover, flavonoids have a chemo preventive role in cancer through their effects on signal transduction in cell proliferation (Weber et al., 1996) and angiogenesis (Fotis et al., 1997). Flavanoids are potent antioxidants and are known to modulate the activities of various enzymes due to their interaction with various biomolecules (Catopano, 1997). Thus antitumoureffecf produced by the MEAL and AEAL may be due to its flavonoids as well as its antioxidant potential. All this findings enable to conclude that MEAL and AEAL possess a protective effect against DAL.

5.2.5 Conclusion

The result of the present investigation is quite encouraging and it explores the potent anticancer activity of Aerva lanata probably because of its direct cytotoxic effect. A decrease in cancer cell count as a confirmatory evidence for protection against DAL, consequently increase in life span was observed with extract treated mice. Hematological parameters and biochemical parameters also enable to conclude on the protective effect of Aerva lanata against DAL. The anti cancer properties of the extracts may be due to the presence of flavanoids, alkaloids and terpenoids. The present study points to the potential anticancer activity of Aerva lanata. However, Further studies are underway to isolate and characterize the active principles responsible for the activity and also pinpoint the mechanism of action are in progress.
Table no. 39 Effect of MEAL and AEAL on the life span, body weight and cancer cell count

<table>
<thead>
<tr>
<th>S.No</th>
<th>Treatment</th>
<th>% ILS</th>
<th>Increase in body weight (gms)</th>
<th>Cell count ml × 10^6</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal control</td>
<td>≥ 30 days</td>
<td>1.32 ± 0.03</td>
<td>_</td>
</tr>
<tr>
<td>2</td>
<td>Cancer control</td>
<td>44 %<strong>(a)</strong></td>
<td>8.50 ± 0.20***(a)**</td>
<td>2.40 ± 0.11</td>
</tr>
<tr>
<td>3</td>
<td>Positive control</td>
<td>88 %</td>
<td>2.24 ± 0.10</td>
<td>0.90 ± 0.09</td>
</tr>
<tr>
<td>4</td>
<td>MEAL (200 mg/ kg)</td>
<td>72 %<strong>(b)</strong></td>
<td>7.20 ± 0.26***(b)**</td>
<td>2.10 ± 0.18**(b)**</td>
</tr>
<tr>
<td>5</td>
<td>AEAL (200 mg/ kg)</td>
<td>76 %<strong>(b)</strong></td>
<td>7.26 ± 0.28***(b)**</td>
<td>1.96 ± 0.12**(b)**</td>
</tr>
</tbody>
</table>

Values are expressed as mean± SEM (n=6) in each group. Values were find out by using one way Anova followed by Newman Keul’s multiple range test. ***(a)*** values were significantly different from normal control at p < 0.001. ***(b)*** values were significantly different from cancer control at p < 0.01.
Table no. 40 Effect of MEAL and AEAL on haematological parameters

<table>
<thead>
<tr>
<th>S.No</th>
<th>Treatment</th>
<th>Total WBC Cells/ ml x 10³</th>
<th>RBC Millions/ cu.mm</th>
<th>Hb gm/ dl</th>
<th>PCV %</th>
<th>Platelets Lakhs/ cu.mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal control</td>
<td>10.25 ± 0.16</td>
<td>4.10 ± 0.20</td>
<td>11.50 ± 0.16</td>
<td>14.39 ± 0.61</td>
<td>2.54 ± 0.21</td>
</tr>
<tr>
<td>2</td>
<td>Cancer control</td>
<td>16.35 ± 0.26***&lt;sup&gt;(a)&lt;/sup&gt;</td>
<td>2.34 ± 0.21***&lt;sup&gt;(a)&lt;/sup&gt;</td>
<td>6.24 ± 0.12***</td>
<td>29.54 ± 0.25***&lt;sup&gt;(a)&lt;/sup&gt;</td>
<td>1.54 ± 0.40***&lt;sup&gt;(a)&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Positive control</td>
<td>11.42 ± 0.20</td>
<td>3.72 ± 0.22</td>
<td>10.22 ± 0.25</td>
<td>18.45 ± 0.24</td>
<td>2.40 ± 0.26</td>
</tr>
<tr>
<td>4</td>
<td>MEAL (200 mg/ kg)</td>
<td>14.2 ± 0.30**&lt;sup&gt;(b)&lt;/sup&gt;</td>
<td>2.72 ± 0.24**&lt;sup&gt;(b)&lt;/sup&gt;</td>
<td>7.45 ± 0.20**&lt;sup&gt;(a)&lt;/sup&gt;</td>
<td>26.20 ± 0.25**&lt;sup&gt;(b)&lt;/sup&gt;</td>
<td>2.15 ± 0.26**&lt;sup&gt;(b)&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>AEAL (200 mg/ kg)</td>
<td>14.6 ± 0.32**&lt;sup&gt;(b)&lt;/sup&gt;</td>
<td>2.86 ± 0.21**&lt;sup&gt;(b)&lt;/sup&gt;</td>
<td>7.50 ± 0.40**&lt;sup&gt;(b)&lt;/sup&gt;</td>
<td>26.41 ± 0.30**&lt;sup&gt;(b)&lt;/sup&gt;</td>
<td>2.19 ± 0.30**&lt;sup&gt;(b)&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n=6) in each group. Values were find out by using one way Anova followed by Newman Keul’s multiple range test. ***<sup>(a)</sup> values were significantly different from normal control at p < 0.001. **<sup>(b)</sup> values were significantly different from cancer control at p < 0.01.
Table no. 41 Effect of MEAL and AEAL on bio chemical parameters and lipid profile.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Treatment</th>
<th>Total Cholesterol (mg/ dl)</th>
<th>TGL (mg/ dl)</th>
<th>AST (IU/ dl)</th>
<th>ALT (IU/ dl)</th>
<th>ALP (IU/ dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal control</td>
<td>110.0 ± 1.83</td>
<td>129.22 ± 1.83</td>
<td>36.84 ± 0.72</td>
<td>30.52 ± 1.13</td>
<td>126.12 ± 1.9</td>
</tr>
<tr>
<td>2</td>
<td>Cancer control</td>
<td>140.90 ± 2.30***&lt;sup&gt;a&lt;/sup&gt;</td>
<td>216.23 ± 1.84***&lt;sup&gt;a&lt;/sup&gt;</td>
<td>84.12 ± 1.61***&lt;sup&gt;a&lt;/sup&gt;</td>
<td>55.13 ± 1.42***&lt;sup&gt;a&lt;/sup&gt;</td>
<td>244.3 ± 2.05***&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>Positive control</td>
<td>122.65 ± 2.20</td>
<td>144.20 ± 2.11</td>
<td>55.47 ± 0.84</td>
<td>41.13 ± 1.10</td>
<td>164.93 ± 1.74</td>
</tr>
<tr>
<td>4</td>
<td>MEAL</td>
<td>131.19 ± 1.03**&lt;sup&gt;b&lt;/sup&gt;</td>
<td>203.70 ± 2.32**&lt;sup&gt;b&lt;/sup&gt;</td>
<td>79.73 ± 0.30**&lt;sup&gt;b&lt;/sup&gt;</td>
<td>51.50 ± 0.54**&lt;sup&gt;b&lt;/sup&gt;</td>
<td>224.88 ± 2.27**&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>AEAL</td>
<td>130.21 ± 1.11**&lt;sup&gt;b&lt;/sup&gt;</td>
<td>197.4 ± 2.45 **&lt;sup&gt;b&lt;/sup&gt;</td>
<td>78.45 ± 0.22**&lt;sup&gt;b&lt;/sup&gt;</td>
<td>50.50 ± 0.45**&lt;sup&gt;b&lt;/sup&gt;</td>
<td>226.70 ± 2.35**&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n=6) in each group. Values were find out by using one way Anova followed by Newman Keul’s multiple range test. ***<sup>a</sup> values were significantly different from normal contro at p < 0.001. **<sup>b</sup> values were significantly different from cancer control at p < 0.01.
Fig. 103 Effect of MEAL and AEAL on the % ILS ;% PCV ; Total WBC cells/ ml $\times 10^3$

I- % ILS ;II- PCV % ; III - Total WBC cells/ ml $\times 10^3$

Fig. 104 Effect of MEAL and AEAL on haematological parameters, increase in body weight and cell count ml $\times 10^6$

I - RBC millions/cu.mm ;II - Hb gm/ dl ; III - Platelets Lakhs/ cu.mm ; IV – Increase in body weight (gms); V – cell count ml $\times 10^6$

Fig. 105 Effect of MEAL and AEAL on biochemical parameters and lipid profile

I- Total Cholesterol, II- TGL, III- AST, IV- ALT, V- ALP
Fig. 106 Normal control

Fig. 107 Cancer control

Fig. 108 Positive control

Fig. 109 MEAL (200mg/ Kg)

Fig. 110 AEAL (200 mg/ Kg)
Histopathological results

Fig. 111 Tumor control (Thigh muscle) Fig. 112 Treated with MEAL (Thigh region)

Fig. 113 Treated with AEAL (Thigh region)

Fig. 111 Section shows skeletal muscle bundle with tumor showing sheets of polygonal to pleomorphic cells with vesicular nuclei. Area of necrosis seen.

Fig. 112 Section shows skeletal muscle bundle and tumor showing small areas of necrosis (30 % of tumor shows necrosis).

Fig. 113 Section shows skeletal muscle bundle and tumor showing small areas of necrosis (30 % of tumor shows necrosis).
Histopathology of Liver

Fig. 114 Normal control

Fig. 115 Tumor control

Fig. 116 Standard

Fig. 117 and 118 Treated with MEAL

A

B

Fig. 119 and 120. Treated with AEAL

A

B
Fig. 114 Section show structure of liver with sheets of hepatocytes separated by sinusoids cartial vein and portal tract appear normal.

Fig. 115 Section shows structure of liver presented hepatic congestion at sinusoids and the portal vessel, pericentre globular micro-steatosis, kuffe cell proliferation, hepatocyte diffuse necrosis and mononuclear infiltrate.

Fig. 116 Section show structure of liver presented mild hepatic congestion at sinusoids and the portal vessel, pericentre globular micro-steatosis, No Kuffe cell proliferation, mild hepatocyte diffuse necrosis and mononuclear infiltrate.

Fig. 117 and 118 Section show structure of liver presented moderate hepatic congestion at sinusoids and the portal vessel, pericentre globular micro-steatosis, less Kuffe cell proliferation, mild hepatocyte diffuse necrosis and mononuclear infiltrate.

Fig 119 and 120 Section show structure of liver presented moderate hepatic congestion at sinusoids and the portal vessel, pericentre globular micro-steatosis, less Kuffe cell proliferation, mild hepatocyte diffuse necrosis and mononuclear infiltrate.
References


Christina AJM, Gladwin JD, Chidambaranathan N. Anticarcinogenic activity of *Withania somnifera* Dunal against Dalton's Ascitic Lymphoma, *J. Ethnopharmacol* 2004; **93**: 359 – 361.


5.3 Comparative evaluation of *in vitro* anthelmintic activity

5.3.1 Introduction

Anthelmintics are drugs that are used to treat infections with parasitic worms. This includes both flat worms, e.g., flukes and tapeworms and round worms, i.e., nematodes. Parasitic worms also infect livestock and crops, affecting food production with a resultant economic impact. Also of importance is the infection of domestic pets. Indeed, the companion animal market is a major economic consideration for animal health companies undertaking drug discovery programmes. Despite the prevalence of parasitic worms, anthelmintic drug discovery is the poor relation of the pharmaceutical industry. The simple reason is that the nations which suffer most from these tropical diseases have little money to invest in drug discovery or therapy. It comes as no surprise therefore that the drugs available for human treatment were first developed as veterinary medicines.

Infection by helminths (worms) may be limited solely to the intestinal lumen or may involve a complex process with migration of the adult or immature worm through the body before localization in a particular tissue. Complicating our understanding of the host parasite relationship and the role of chemotherapy in helminth induced infections is the complex life cycle of many of these organisms. Whereas some helminths have a simple cycle of egg deposition and development of the egg to produce a mature worm, others must progress through one or more hosts and one or more morphological stages, each metabolically distinct from the other, before emerging as an adult. Furthermore, an infective form may be either an adult worm or an immature worm. Treatment may be further complicated by infection with more than one genus of helminth. Pathogenic helminths can be divided into the following major groups: cestodes (flat worms), nematodes (round worms), trematodes (flukes) and less frequently, Acanthocephala (thorny headed worms).

Throughout the world, the parasitic helminthic infection increases the mortality and morbidity day by day. This includes the intestinal nematodes eg hook worms (*Ancylostoma duodenale*), roundworms (*Ascaris lumbricoids*), trematodes eg. Flukes (*Schistosoma mansoni* and *Schistosoma hematobolium*) and cestodes eg.Tapeworms (*Taenia solium*). It is
unevenly distributed disease in low income countries which affected worstly and highest risk of morbidity because it is the major source of environmental contamination and transmission. Piperazine, albendazole, mebendazole and praziquantel are the commonly used drugs acting as anthelmintics having broad spectrum activity and high cure rates due to the sustainability of the periodic emergence of resistance. It is in the context that the people consume several plants or plant derived preparations to cure helminthic infections (Satyavathi, 1990).

a) Roundworms

The migration of the larval forms and eggs transmission through skin contact in moist soil and in tropical areas causes migraine, eosinophilia and pulmonary related problems. The common infections occurring with intestinal worms include *Ascaris lumbricoides*, *Trichuris trichiura*, *Necator americanus* and *Ancylostoma duodenal* with the household aggregation of infection. The eggs are deposited on perianal area that is due to self infection. These infections also occur due to the contaminated surfaces like carpets, curtains etc. The airborne and inhalation of the small number of eggs are transmitted through ingestion of the infected food because the humans are the accidental hosts. After the ingestion of the infected products the immunological lungs, liver and central nervous system damages occur.

b) Flukes

Flukes are the parasitic trematodes of *Schistosoma* species which are transmitted through direct contact with fresh water. They penetrate into the intact human skin and enter the capillaries and then migrate to the central and portal system where they mature. Acute schistosomiasis also known as Katayama fever, which is a form of visceral larval migraines. The adult male and female pairs ultimately migrate to the superior mesenteric veins and ureteric vesicles. The eggs are then shed in the faeces and urine.

c) Tapeworms

Humans are the intermediate host for the *Taenia solium* with the development of the tissue cysts. After the ingestion of the uncooked beef (*T. saginata*) or pork it develops the cysts and it causes the mild abdominal symptoms. The infestations of the central nervous systems
caused due to the pork tapeworm or flukes are known as neurocysticercosis which is treated through albendazole and praziquantel. The complex life cycle and host parasite relationship means that treatment is sometimes difficult and may have to be protracted. Most available anthelmintic drugs exert their antiparasitic effects by interference with energy metabolism, neuromuscular coordination, microtubular function, cellular permeability. Some of the drugs used in the treatment of diseases caused by helminths are also used in the treatment of specific protozoal diseases. Some of the nematodes (filarial worms and guinea worms) live in blood, lymphatics and other tissues and are referred to as blood and tissue nematodes. They are found primarily in the intestinal tract. One group, hook worms, undergoes a development cycle in soil. The larvae penetrate the skin of humans, enter the venules and are carried to lungs, where they enter the alveoli, some times causing pneumonitis. The larvae then migrate up the trachea and are swallowed. In the intestine, they attach to the mucosa, and using the cutting plates and a muscular oesophagus, feed on host blood and tissue fluid. This may result in vague abdominal, pains, diarrhoea and if many worms are present anaemia. Strongyloides stercoralis infection is acquired, like hook worm, from filariform larvae in contaminated soil that penetrate the skin. This parasite maintains itself for many decades in the small intestine asymptomatically. Persons treated with immunosuppressive drugs or who are deliberated by chronic illness may be at risk for widespread tissue invasion or hyperinfection syndrome. Prompt treatment may be life saving in disseminated disease. Other intestinal nematodes are acquired by ingestion of eggs from soil. These group lack cutting plates and may not cause anaemia. Still other nematodes such as pinworms migrate from the anus to lay eggs, which are transmitted by fingers or through the air. The eggs are ingested and the adult worm develops in the intestinal tract. In some cases, the appendix may be invaded, resulting in symptoms are perianal pruritus and a restlessness associated with the migration of the female worm through the anus to the perianal skin. Other nematodes, such as Ascaris spp, are ingested in egg form but have a migration similar to that of the hook worm. The filarial worms differ from other nematodes in that they are thread like and are found in blood and tissue. The infective larvae enter
following the bite of an infected anthropod (fly or mosquito). Then they enter the lymphatics and lymph nodes. Fever, lymphangitis and lymphadenitis are associated with the early stage of the disease. Chronic infections may be characterized by elephantiasis as a result of lymphatic obstruction. Some species of filarial worms migrate in the subcutaneous tissues and produce nodules and blindness (onchocerciasis). Most of the screenings reported are in vitro studies using some worm samples like Indian earthworm *Pheretima posthuma*, *Ascardia galli*, *Ascaris lumbricoids*, etc.

Nature has provided a complete store-house of remedies to cure all ailments of mankind and its related diseases. The human being appears to be affected with more diseases than any other animal species. There can be little doubt then that is sought out to alleviate his suffering from injury and disease by taking advantage of plants growing around him. In the past, almost all the medicines used were extracted from the plants and the plant being man's chemist for ages. Medicinal plants are the source of great economic value in the Indian subcontinent. Herbal medicine is still the main source of medicine and about 75 - 80 % of the whole population, mainly in developing countries for primary health care because of better cultural acceptability, better compatibility, with the human body and fewer side effects. Most diseases caused by helminths are chronic, debilitating in nature, they probably cause more morbidity, greater economic and social deprivation among humans and animals than any other parasites. It has been estimated that about half of the world’s population suffers from *Helminthiasis* and the number is increasing day by day. It is not only limited to tropical and subtropical countries but is also to endemic in many regions because of poor sanitation, poor family hygiene, malnutrition and crowded living condition (Mohammed *et al*, 2005). Potent anthelminitics are available today, and treatment is frequently done by using different types of drugs. However the high costs of modern anthelmintics have limited effective control of the parasites. In some cases, wide spread use of low quality anthelmintics are used for the development of resistance and hence causes reduction in use of anthelmintics. In the recent years, the importance of herbal drugs in medicine has tremendously increased because of their fewer side effects. Consequently, the demand for the herbal formulation is increasing.
day by day. The phytochemical constituents and their standardization are accelerated with the development of instrumental analysis and this field becomes important and new for investigation. As the half of world suffering from bacterial and helminthes infection, the source of infection being very common due to poor sanitation, poor family hygiene, malnutrition, and crowded living conditions.

The sources of infections are

1) Human being: The commonest source of infection is human being themselves.

2) Animals: Many pathogens are able to infect both human being and animals. Animals act as source of human infection.

3) Insects: Blood sucking insect may transmit pathogen to human beings. Besides acting as vector, some insect may also act as a reservoir for hosts.

4) Soil and water: Some pathogens can survive in the soil for very long periods. Water may act as the source of infection either due to contamination with pathogenic microorganism or due to presence of aquatic vector.

5) Food: Contaminated food act as a source of infection(Ananthnarayan and Thomson,2000). So there is a need to develop anthelmintics drug from herbal source.

The use of medicinal plant is growing worldwide because of the increasing toxicity and allergic manifestations of the synthetic drugs. Helminth infections are among the most common infections in man, affecting a large proportion of the world’s population. In developing countries, they pose a large threat to public health and contribute to the prevalence of malnutrition, anaemia, eosinophilia and pneumonia. The disease is highly prevalent particularly in third world countries (Dhar et al., 1982) due to poor management practices. Although the majority of infections due to worms are generally limited to tropical countries, they can occur to travellers, who have visited those areas and some of them can be developed in temperate climates (Bundy, 1994). However, increasing problems of development of resistance in helminths (Coles, 1997) against anthelmintics have led to the proposal of screening medicinal plants for their anthelmintic activity. Because of limited availability and affordability of modern medicines, most of the world’s population depends to
a greater extent on traditional medical remedies. The traditional medicines hold a great promise as source of easily available effective anthelmintic agents to the people, particularly in tropical developing countries, including India.

In the treatment of parasitic diseases, the anthelmintic drugs are used indiscriminately. Ideally an anthelmintic agent should have broad spectrum of action, with high percentage of cure with a single therapeutic dose, free from toxicity to the host and should be cost effective. None of the synthetic drug available meets this requirement. Even most common drugs like Piperazine salts have been shown to have side effects like nausea, intestinal disturbances and giddiness (Liu et al., 1996). Resistance of the parasites to existing drugs (Walter et al., 1985) and their high cost warrants the search for newer anthelmintic molecules. The origin of many effective drugs is found in the traditional medicine practices and in view of this several researchers have undertaken studies to evaluate folklore medicinal plants for their proclaimed anthelmintic efficacy. Recently the use of anthelmintic produces toxicity in human beings. Hence the development and discovery of new substances acting as anthelmintics are being derived through plants which are considered to be the best source of bioactive substances.

The aerial parts of *Aerva lanata* used as anthelmintic traditionally. Literature survey reveals that there are no reports on systematic and scientific study of anthelmintic activity of has been reported, an attempt has been made to evaluate the anthelmintic potential of aerial parts of *Aerva lanata*.

5.3.2 MATERIALS AND METHODS

5.3.2.1 Drug and chemicals

The drug piperazine citrate (Glaxo Smithkline Pharmaceutical Ltd) purchased from commercial sources and all other chemicals were of analytical grade.

5.3.2.2 Evaluation of anthelmintic activity

Anthelmintic activity was carried as per the method reported by (Ajaiyeoba et al, 2001) with minor modifications. The assay was performed on adult Indian earth worm *Pheritima posthuma* due to its anatomical and physiological resemblance with the intestinal round
worm parasite of human beings (Vidyarthi, 1967; Vigar, 1984; Thorn et al., 1977; Chatterjee, 1967). Because of easy availability earthworm have been widely used for the initial evaluation of anthelmintic compounds in vitro (Sollmann, 1918; Jain and Jain, 1972; Dash et al., 2002; Shivkar and Kumar, 2003). Fifty millilitre of formulation containing three different concentrations, each of crude methanol and aqueous extracts (25, 50, 100 mg/ml in Tween 80/normal saline). This was done in duplicate for both the extracts. All the extracts and the standard drug solution were freshly prepared before starting the experiments. Mean time for paralysis (in min) was noted when no movement of any sort could be observed except when the worm was shaken vigorously; time for death of worms (in min) was recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50°C). Piperazine citrate was prepared by dissolving in normal saline at a concentration of 15 mg/ml which was used as reference standard (Mali et al., 2005).

5.3.3 Results and discussions

Preliminary phytochemical studies on Aerva lanata revealed the presence of flavanoid glycosides, steroids, carbohydrates, alkaloids, tannins, proteins and flavanoids. Some of these phytoconstituents may be responsible to show a potent anthelmintic activity. From the result both methanol and aqueous extract of the aerial parts of Aerva lanata show an anthelmintic activity when compared to the standard drug. Each MEAL and AEAL at the concentration of 25, 50 and 100 mg/ml produced anthelmintic activity in dose dependent manner giving shortest time of paralysis (P) and death (D) with 100 mg/ml concentration (Fig. 121). MEAL at the concentration of 100 mg/ml caused paralysis in 7.5 min and death in 11.16 min, while AEAL showed paralysis in 13.83 min and death in 18 min against Pheritima postuma. The reference drug piperazine citrate showed the same at 14.16 min and 31.83 min respectively (Table no. 42). The predominant effect of piperazine citrate on the worm is to cause a flaccid paralysis that result in expulsion of the worm by peristalsis. Piperazine citrate by increasing chloride ion conductance of worm muscle membrane produces hyper polarisation and reduced excitability that leads to muscle relaxation and flaccid paralysis (Martin, 1985). Phytochemical analysis of the crude extracts revealed the
presence of tannins as one of the chemical constituents. Tannins were shown to produce anthelmintic activities (Niezen et al., 1995). Chemically tannins are polyphenolic compounds (Bate smith, 1962). Some synthetic phenolic anthelmintics (eg) niclosamide, oxyclozanide and bithionol are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation (Martin, 1997). It is possible that tannins contained in the extracts of Aerva lanata produced similar effects. Tannins, the secondary metabolite, occur in several plants have been reported to show anthelmintic property by several investigators (Waller et al., 1997). Tannins, the polyphenolic compounds, are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation (Martin, 1997) or, binds to the glycoprotein on the cuticle of parasite (Thompson and Geary, 1995) and cause death. Coming to the chemistry of nematode surface, it is a collagen rich extracellular matrix (ECM) providing protective cuticle that forms exoskeleton, and is critical for viability, the collagen is a class of proteins that are modified by a range co-and post –translational modification prior to assembly into higher order complexes (or) ECMS. The mammalian skin also consists largely of collagen in the form of fibrous bundles. In leather making industry, vegetable tannins are commonly used in the tanning operation of leather processing that imparts stability to collagen of skin matrix through its reactivity and hence make the collagen molecule aggregate into fibres. This results in the loss of flexibility in the collagen matrix and gain of mechanical property with improved resistance to the thermal (or) microbial/enzymatic attack. Similar kind of reaction is expected to take place between the nematode cuticle (the earth worm) and the tannin of Aerva lanata, possibly by linking through hydrogen bonding, as proposed in this study. This form of reactivity brings toughness in the skin and hence the worms become immobile and non-functional leading to paralysis followed by death. Hence further investigation and proper isolation of the active principles might help in the findings of new lead compounds, which will be effective against various parasitic infections.
The another possible anthelmintic effect of tannins is that they can bind to free proteins in the gastro intestinal tract of host animal (Athnasiadou et al., 2001) or glycoprotein on the cuticle of the parasite (Thompson and Geary, 1995) and cause death.

5.3.4 Conclusion

Ancient classical literature and ethnomedical surveys described the use of plants in traditional system of medicines for the treatment of Helminthic infections. This traditional medical wisdom is excellent proof of clinical efficacy and safety of medicinal plants. The present report is the invitro studies of aerial parts of *Aerva lanata* Linn Juss.

In conclusion, the traditional claim of aerial parts of *Aerva lanata* as an anthelmintic has been confirmed as the extracts shown activity against *Pheritima postuma*. At the same time efforts should be made to standardize the plant extracts with good anthelmintic activity and formulate best alternative herbal preparations to replace or complement the synthetic drugs which are currently in use.
Table no. 42 Anthelmintic activity of methanol and aqueous extracts of aerial parts of *Aerva lanata* Linn Juss

<table>
<thead>
<tr>
<th>S. no</th>
<th>Treatment drug</th>
<th>Concentration used</th>
<th>Time taken for paralysis (min)</th>
<th>Time taken for death (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Piperazine citrate</td>
<td>15 mg/ ml</td>
<td>14.16 ± 0.6009</td>
<td>31.83 ± 0.6540</td>
</tr>
<tr>
<td>2</td>
<td>MEAL</td>
<td>25 mg/ ml</td>
<td>26.66 ± 0.4944</td>
<td>34.83 ± 0.6009</td>
</tr>
<tr>
<td>3</td>
<td>MEAL</td>
<td>50 mg/ ml</td>
<td>18.83 ± 0.7031</td>
<td>22.33 ± 0.5577</td>
</tr>
<tr>
<td>4</td>
<td>MEAL</td>
<td>100 mg/ ml</td>
<td>7.5 ± 0.5627</td>
<td>11.16 ± 0.4772</td>
</tr>
<tr>
<td>5</td>
<td>AEAL</td>
<td>25 mg/ ml</td>
<td>32.5 ± 0.6191</td>
<td>40.33 ± 0.8432</td>
</tr>
<tr>
<td>6</td>
<td>AEAL</td>
<td>50 mg/ ml</td>
<td>24.83 ± 0.7923</td>
<td>28 ± 0.7745</td>
</tr>
<tr>
<td>7</td>
<td>AEAL</td>
<td>100 mg/ ml</td>
<td>13.83 ± 0.6540</td>
<td>18 ± 0.6831</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n=6)
Fig. 121 Anthelmintic activity of MEAL and AEAL

Each bar is represented as mean ± standard deviation (n=6).

Group I – Control (Normal saline), group II - standard (Piperazine citrate), group III to V- Methanol extract of dose 25, 50, 100 mg/ml respectively and group VI to VIII- Aqueous extract of dose 25, 50, 100 mg/ml respectively.
References


