Preventive and Curative Potential of *Vigna mungo* against Metabolic Syndrome in Acute and Chronic Rat Models

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**A B S T R A C T**

The modern lifestyle of high intake of high-calorie food associated with reduced energy expenditure plays an important role on the increased prevalence of type 2 diabetes. There is a multiplicity of effective treatment choices with synthetic drugs, but they have a number of side effects. *Vigna mungo* (Black gram), while acting as one of the richest sources of proteins and amino acids, has high fiber content which could enhance the lipoprotein lipase activity. The present study evaluates the antihyperlipidemic and antihyperglycemic potential of *V. mungo* in triton and high-fat diet in combination with low dose streptozotocin (HFD+STZ) rat models. Orally administered *V. mungo* extract (VME) at a dose 100 mg kg\(^{-1}\) in both acute and chronic rat models significantly lowered levels of triglycerides, LDL and total cholesterol and significantly increased HDL levels in comparison to triton and HFD+STZ induced rats. Glucose level analysis on the blood plasma level suggested that VME at 100 mg kg\(^{-1}\) shows significant reduction of 34.90% in glucose level on HFD+STZ induced rats. The determination of Oral Glucose Tolerance Test (OGTT) showed that VME at 100 mg kg\(^{-1}\), shows significant improvement in glucose tolerance to exogenously administered glucose (2 g kg\(^{-1}\)). The results observed for biochemical analysis, blood glucose level and OGTT support the medicinal value of *V. mungo* as a potential source having antihyperlipidemic and antihyperglycemic properties for the prevention and treatment of type 2 diabetes.

**Key words:** Black gram, diabetes, hyperlipidemia, hyperglycemia, high fat diet

**INTRODUCTION**

Metabolic syndrome is a clinical condition associated with various complications including hyperlipidemia, hyperglycemia, hypertension (Huang, 2009; Kaur *et al*., 2015), abdominal obesity and insulin resistance (Rivera *et al*., 2008). It has become a progressively prevalent disorder that parallels the worldwide epidemic of obesity and type 2 diabetes (Weiss *et al*., 2004). These metabolic syndromes are associated with an augmented risk of developing cardiovascular diseases (Eckel *et al*., 2005) that are the main cause of premature mortality in type 2 diabetes (Reaven, 1995).

Current epidemiological data shows that almost 215 million individuals worldwide, suffer from diabetes and 80-90% of them suffer from type 2 diabetes (Narender *et al*., 2006). The sedentary lifestyle of increased intake of high-calorie food along with decreased energy usage, contributes to the present growing prevalence of type 2 diabetes and obesity (Aude *et al*., 2004). According to recent epidemiological studies, approximately 90% of all the people with type 2 diabetes are or have been overweight and signified that obesity is a major cause and a risk factor of type 2 diabetes and related metabolic disturbances (Bray and Bellanger, 2006; Kahn *et al*., 2006). To reduce complications and negative consequences of metabolic syndrome, the control of blood glucose and lipids is necessary (Moller, 2001). Existing therapeutic choices such as dietary modification or a multi-regimen of synthetic antidiabetic drugs have their own limits and undesirable side effects (Lender and Sysko, 2006).
Because of this, new medicinal agents with properties on monitoring both lipids and blood glucose are in excessive demand (Nammi et al., 2009).

Daily intake of pulses is suggested to human to reduce risk of cardiovascular diseases, digestive tract diseases, obesity, etc. (Duranti, 2006). Vigna mungo, commonly known as black gram, is an excellent pulse having a composition of essential constituents such as vitamins, proteins, carbohydrates, flavonoids, phenolic compounds, saponins, etc. (Patidar et al., 2012). These constituents can be very beneficial in faster removal of free fatty acids from circulation, which causes a reduction in total cholesterol by increasing the lipoprotein lipase activity (Solanki and Jain, 2010). The extract of V. mungo (VME) having low glycemic index and high fibre content is helpful in the treatment of obesity and type 2 diabetes (Meenu et al., 2011).

Triton WR1339, also known as tyloxapol, is nonionic detergent and is used by numerous studies to induce acute hyperlipidemia and hyperglycemia in animals (Harnafi et al., 2008; Bertges et al., 2011). It inhibits the lipoprotein lipase activity that causes accumulation of Very Low Density Lipoprotein (VLDL) and triglycerides in plasma (Zarzecki et al., 2014). It also, stimulates HMG-CoA reductase activity that further causes significant increase in biosynthesis of hepatic cholesterol (Zarzecki et al., 2014).

High Fat Diet (HFD) in combination with low dose streptozotocin (STZ) model proves to be a better choice to induce type 2 diabetes (Zhang et al., 2008). The HFD-fed rats show significant increase in basal plasma glucose, body weight, insulin, total cholesterol and triglycerides levels (Srinivasan et al., 2005). Also, these insulin resistant HFD-fed rats develop hyperglycemia upon STZ injection (Srinivasan et al., 2005). The HFD with STZ rats (HFD+STZ) offers a novel animal model for type 2 diabetes by simulating the human syndrome and is therefore, appropriate for the testing of antidiabetic drugs (Reed et al., 2000).

Therefore, the present study was done to evaluate preventive and curative potential of V. mungo against metabolic syndrome in triton rat model and HFD+STZ rat models.

MATERIALS AND METHODS

Plant material and authentication: The seeds of V. mungo were obtained from the local market of Mumbai, India. Samples, vouchers and specimens were preserved.

Preparation of plant extract and dose: Seeds of the V. mungo were ground by electrical grinder to a fine powder. Dried fine powder of plant drug was defatted with 60% petroleum ether to remove fatty materials and other pigmentation. Powder was dried and again used for extraction with ethanol by soxhlet extraction process. Ethanolic content was removed by distillation while water content was removed by using Rotary evaporator. The yield of the extract was 6-8% w/w and stored at 2-8°C in refrigerator for further use during experiment.

Standardization of plant material and phytochemical screening of VME: The standardization of the obtained plant material was done by studying the quality and purity of the obtained material. The extract of V. mungo was tested for the presence or absence of different secondary metabolites like carbohydrates, steroids, flavonoids, proteins, glycosides and phenolic compounds with the aid of different standard chemical tests (Khandelwal, 2005; Kokate, 1997).

Chemicals: The following reference chemicals were obtained from the sources specified: Triton WR1339 (Tyloxapol) and STZ from SRL laboratories, diethyl ether (Sigma-Aldrich Chemie GmbH, Germany) and high fat diet. Commercial ERBA diagnostic kits for serum analysis. Triglyceride Estimation Kit, Total Cholesterol Estimation Kit, low density lipoprotein direct kit, high density lipoprotein direct kit, oral glucose tolerance test and glucose oxidase kit were procured from Transasia Bio-medicals Ltd, Mumbai, India. All chemicals used were of the highest purity grade.

Experimental animals: Albino Wistar rats weighing 150-200 g obtained from animal house of SPP-SPTM NMIMS were used in the experiment. The animals were housed in polycarbonate cages at room temperature (25±2°C) and humidity (75±5%) with 12:12 h light-dark cycle. The animals were acclimatized for one week before starting experimental work. Animals used in the present study were approved by the Institutional animal ethics committee, formed as per the guidelines of committee for the purpose of control and supervision on experiments on animals. All the studies were started after obtaining prior approval from the Institutional Animal Ethical Committee in accordance (IAEC) (Approval No: CPCSEA/IAEC/SPTM/P-04/2013).

Acute toxicity studies: Albino Wistar rats of either sex were randomly divided into two groups (control and test), each containing three animals. The extract was administered orally to the test group at doses of 2000 mg kg⁻¹ (OECD guidelines 423). Distilled water was administered to the control group. The general behavior of rats was continuously observed for 1 h after dosing, periodically during the first 24 h with special attention given during the first 4 h and daily thereafter, upto the 14 days. Changes in the normal activity of rats and their body weights were monitored and the time at which signs of toxicity or death appeared recorded.

Acute model: Evaluation of hypolipidemic, hypcholesterolemic and hypotriglyceridemic potentials of the VME in comparison to Atorvastatin was carried out by inducing acute hyperlipidemia, using Triton WR1339 in rats.
Experimental animal groups: Hyperlipidemia was induced in Wistar albino rats by single intraperitoneal injection of freshly prepared solution of Triton WR1339 (300 mg kg\textsuperscript{-1}) in physiological saline solution after overnight fasting for 18 h. The animals were divided into four groups, each group consisting of six animals. The experimental design and schedule of treatment was followed as:

- **Group I:** Control group
- **Group II:** Triton WR1339 induced hyperlipidemic rats
- **Group III:** Triton WR1339 induced hyperlipidemic rats treated with standard atorvastatin (10 mg kg\textsuperscript{-1} orally) simultaneously with triton injection and 24 h later
- **Group IV:** Triton WR1339 induced hyperlipidemic rats treated with extracts of Pioglitazone group and VME group *V. mungo* (100 mg kg\textsuperscript{-1} orally). The animals were pre-treated with the extracts for 7 days

Biochemical analysis in serum: Blood samples were taken before and after 24 and 48 h of administration of triton. The samples were stored and serum was separated by centrifuging at 4000 rpm for 10 min and stored at -20°C prior to analysis. Biochemical parameters such as total cholesterol, triglyceride (TG), Low Density Lipoprotein (LDL) and High Density Lipoprotein (HDL) values were determined using ERBA Chem-7 Trans Asia diagnostic kits.

Chronic model: Evaluation of hypolipidemic, hypocholesterolemic and hypotriglyceridemic potentials of the VME in comparison to pioglitazone was carried out with the help of HFD in combination with low-dose STZ induced type 2 diabetes rat model (Table 1).

Experimental animal groups: The animals were divided into four groups, each group consisting of six animals. The design of experiment and schedule of treatment was as follows:

- **Group I:** Control group
- **Group II:** Rats received HFD and a single dose of 30 mg kg\textsuperscript{-1} of STZ by intraperitoneal route. Blood glucose level above 200 mg dL\textsuperscript{-1} was considered to be diabetic
- **Group III:** Rats received HFD with STZ 30 mg kg\textsuperscript{-1} and were treated with standard drug pioglitazone 10 mg kg\textsuperscript{-1} orally
- **Group IV:** Rats received HFD with STZ 30 mg kg\textsuperscript{-1} and were treated with VME 100 mg kg\textsuperscript{-1} orally

Biochemical analysis in serum: Blood samples were taken before treatment and after 0, 14 and 28 days of administration of STZ. The serum samples were separated by centrifuging at 4000 rpm for 10 min and stored at -20°C prior to analysis. The parameters like total cholesterol, TG, LDL and HDL were estimated using ERBA Chem-7 Trans Asia diagnostic kits. The blood glucose level was determined and recorded in the table and the graph of glucose concentration vs. time period was plotted. The antidiabetic activity of *V. mungo* was compared with that of standard drug pioglitazone.

Estimation of Oral Glucose Tolerance Test (OGTT): This test was carried out on the 28 day of treatment; all the groups were fasted for 16 h before giving an oral glucose load (2 g kg\textsuperscript{-1}). The blood was withdrawn from the retro orbital sinus at -30, 0, 15, 30, 60, 90, 120 and 180 min after the glucose load from all the animals for the estimation of glucose. The levels of fasting blood glucose were estimated by the procedure as per the manufacturer of the kit (Erba Diagnostics, Germany).

Statistical analysis: The differences among experimental and control groups were determined using the Graph Pad INSTAT 5.0.3.477 software for Windows. Comparisons among different groups were performed by analysis of variance using ANOVA test. Significant difference between control and experimental groups were assessed by student’s t-test.

RESULTS

Preliminary phytochemical screening: The test results for VME extract indicated the presence of carbohydrates, steroids, flavonoids, proteins, glycosides and phenolic compounds.

Acute toxicity studies: The VME was found to be non-toxic up to the dose of 2 g kg\textsuperscript{-1} and did not cause any mortality or symptoms of toxicity through the 14 days period. As per guidelines given by Organization for Economic Cooperation and Development (OECD guidelines 423) for acute oral toxicity, a Lethal Dose 50% (LD\textsubscript{50}) of 2000 mg kg\textsuperscript{-1} and above is labeled as “unclassified” and hence *V. mungo* is found to be safe. Therefore, further dosing to find out LD\textsubscript{50} of VME was not performed (Table 2).

Acute model: As seen in Fig. 1, the total cholesterol, TG and LDL in atorvastatin group and pre-treated VME group were

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg kg\textsuperscript{-1})</th>
<th>Sign of toxicity (ST/NB)</th>
<th>Mortality (D/S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (control)</td>
<td>0</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>II (VME)</td>
<td>2000</td>
<td>0/3</td>
<td>0/3</td>
</tr>
</tbody>
</table>

ST: Sign of toxicity, NB: Normal behavior, D: No. of deaths, S: Survived, VME: *V. mungo* extract

Table 1: Atherogenic diet composition

<table>
<thead>
<tr>
<th>Components</th>
<th>Amount (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat chow</td>
<td>72.1</td>
</tr>
<tr>
<td>Milk powder</td>
<td>3.5</td>
</tr>
<tr>
<td>Salt</td>
<td>1.0</td>
</tr>
<tr>
<td>Multivitamins</td>
<td>0.1</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>1.5</td>
</tr>
<tr>
<td>Hydrogenated fat</td>
<td>16.7</td>
</tr>
<tr>
<td>Coconut oil</td>
<td>5.0</td>
</tr>
</tbody>
</table>

Table 2: Signs of toxicity and mortality results of acute toxicity study of *V. mungo* extract

Biochemical parameters

Fig. 1: Effect on biochemical parameters in triton induced model. All the groups were significant (p<0.001) when compared to triton treated group

Fig. 2: Effect on biochemical parameters in serum of HFD+STZ model. All the groups were significant at p<0.001, p<0.01 and p<0.05 compared to triton induced treatment

observed to be significantly (p<0.001) decreased as compared to triton induced group whereas HDL values in atorvastatin group and pre-treated VME group were observed to be significantly (p<0.001) increased as compared to triton induced group. Furthermore, there was no significant difference in the effect of atorvastatin and VME. Therefore, in comparison to triton induced group, with the pre-treatment of VME 100 mg kg\(^{-1}\), significant reduction of total cholesterol by 42.29%, TG by 74.32% and LDL by 21.52% were observed.

Chronic model

Effect of biochemical parameters in serum: As seen in Fig. 2, the total cholesterol, TG and LDL in pioglitazone group and VME group were observed to be significantly decreased as compared to HFD+STZ group whereas HDL values in pioglitazone and VME groups were observed to be significantly increased as compared to HFD+STZ group. It was also observed that the effect of VME on total cholesterol (p<0.001) was more significant than that of pioglitazone (p<0.01). Therefore, in comparison to HFD+STZ group, with the treatment of VME 100 mg kg\(^{-1}\), a significant reduction of total cholesterol by 48.07%, TG by 38.50% and LDL by 44.40% were observed, whereas HDL values significantly increased by 83.46%.

Effect on blood glucose level: On the 14th day of treatment, the level of blood glucose was reduced by 27.99% in pioglitazone group and by 10.51% in VME group in comparison to HFD+STZ group. However, after 28 days of treatment, VME group showed more effect on blood glucose (significant reduction by 34.90%) than that of pioglitazone group (significant reduction by 34.54%) in comparison to HFD+STZ group (Fig. 3).

Effect on oral glucose intolerance test: As seen from Fig. 4, OGTT revealed that low-dose STZ with HFD group, causes an impairment of tolerance to glucose after giving oral glucose load (2 g kg\(^{-1}\)) because glycemic levels raised at 15, 30, 60, 120 and 180 min in comparison to control group. Treatment with Pioglitazone 10 mg kg\(^{-1}\) and VME 100 mg kg\(^{-1}\) significantly decreased glucose levels in HFD+STZ group by 28.99 and 22.49%, respectively.

DISCUSSION

Metabolic syndrome is a complex polygenic disorder resulting in part from the contribution of impaired insulin
secretion and/or impaired insulin action on its receptors (Roche et al., 2005). Fats become the preferred source of energy, when carbohydrates are in less quantity, or when their breakdown is not complete (Reddy et al., 2009). Due to this, fatty acids are conducted into the general circulation, giving rise to secondary triglyceridaemia where the total cholesterol, triglycerides, serum lipids and phospholipids intensify, leading to life-threatening disorders of lipid (Narender et al., 2007).

The development of metabolic syndrome is influenced by a combination of genetic and environmental factors (Despres and Lemieux, 2006). Among all the environmental influences, long-term high-fat intake is mostly studied for its involvement with the development of metabolic syndromes in rodents and human beings (Kim et al., 2004). A great proportion of day-to-day energy derived from fat component is becoming a common habit in modern day lifestyle of the world (Nammi et al., 2009). The high prevalence of metabolic disorders is probably related to abnormal blood lipid profiles probably due to long term effects of high fat intake (Isomaa et al., 2001).

Many drugs available for the treatment of metabolic disorders like hyperlipidemia and diabetes, but these drugs have their own side effects. For example, popular drugs like atorvastatin has serious side effect such as myopathy (Moosmann and Behl, 2004), whereas, pioglitazone can cause bone loss (Shah and Mudaliar, 2010). Because of this, new natural medicinal agents with properties on monitoring bone loss (Shah and Mudaliar, 2010). Because of this, new natural medicinal agents with properties on monitoring bone loss (Shah and Mudaliar, 2010). Therefore, in the chronic study it was used as the acute model to investigate the preventive effect of V. mungo against metabolic syndrome. Therefore, in the chronic study it was used as the acute model to investigate the preventive effect of V. mungo against metabolic syndrome. The results in Atorvastatin group and pre-treated VME group of biochemical parameters like total cholesterol, TG and LDL were observed to be significantly (p<0.001) decreased whereas HDL values were observed to be significantly (p<0.001) increased as compared to triton induced group. There was no significant difference in the effect of Atorvastatin and VME. This suggests that the V. mungo can be as effective as Atorvastatin without having to deal with side effects like myopathy that can be caused by atorvastatin. Therefore, in comparison to triton induced group, with the pre-treatment of VME 100 mg kg⁻¹, significant reduction of total cholesterol by 42.29%, TG by 74.32% and LDL by 21.52% were observed, whereas HDL values significantly increased by 61.32%.

The results of HFD+STZ chronic study in pioglitazone group and VME group for biochemical parameters like total cholesterol, TG and LDL were observed to be significantly decreased, whereas HDL values were observed to be significantly increased as compared to HFD+STZ group. It was also observed that the effect of VME for total cholesterol (p<0.001) was more significant than that of pioglitazone (p<0.01). Therefore, in comparison to HFD+STZ group, with the treatment of VME 100 mg kg⁻¹, reduction of total cholesterol by 48.07%, TG by 38.50% and LDL by 44.40% were observed. The chronic study suggests that VME can be more effective than a popular drug like pioglitazone and it is hypothesized that it acts by increasing the utilization of cholesterol in our body.

On the administration of VME 100 mg kg⁻¹, the values of blood glucose level decreased significantly. Therefore, it can be hypothesized that V. mungo components as vitexin, proteins, carbohydrates, flavonoids, phenolic compounds, saponins with its low glycemic index helped in lowering the glucose values in HFD-STZ rats.

Further, the condition for the predictive diagnosis of type 2 diabetes is based on oral glucose intolerance test. Treatment with VME at doses 100 mg kg⁻¹ significantly decreased plasma glucose levels by 22.49% in HFD and low dose streptozotocin treated diabetic rats. The results of above studies clearly indicate the potential of V. mungo herbal extract in the prevention and treatment of metabolic syndrome.

CONCLUSION

Based on the current study, it is concluded that V. mungo possesses significant protective and curative properties against metabolic syndrome caused by Triton WR1339 induced hyperlipidemia and HFD+STZ induced diabetes mellitus. It can be concluded that the extract showed both hypolipidemic and hypoglycemic effect so that it can be used in the treatment of the metabolic syndrome.
REFERENCES


