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Research Article

Evaluation of antidiabetic potential of *Vitis vinifera* leaves extracts in acute and chronic animal models

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ABSTRACT
The objective of the present study is to evaluate antidiabetic potential of *Vitis vinifera* leaves extracts. The quantitative and qualitative screening of prepared *Vitis vinifera* methanolic (VVME) and aqueous (VVAE) extracts was carried out by employing various phytochemical tests. The antidiabetic activity was evaluated by using OGTT (acute study), alloxan (150 mg/kg, *i.p.*) (sub-acute study) and streptozotocin (55 mg/kg, *i.p.*) (chronic study) induced diabetes in rats. Diabetes was assessed by measuring the serum blood glucose level in experimental animals at different doses i.e. 100, 200 and 400 mg/kg of VVME and VVAE. Hyperglycemia and hyperlipidemia were found to be significantly attenuated by different doses of both extracts, however methanolic was found to be more effective. It is clear with the present study that grape leaves can be used as antidiabetic agent and good functional food option for both food and pharmaceutical industries.

KEYWORDS
Vitis vinifera, antidiabetic, streptozotocin, alloxan etc.

1. INTRODUCTION

The medicinal plants used as a source of remedies to cure various types of diseases of mankind. So, majority of population of world largely relies on plants store house owing to its natural origin and lesser side effects.[1] Grape is a large deciduous climber, climbing by means of intermittent, leaves opposed, large, often bifid tendrils, cultivated in many part of India.[2] Grape has been used in folk medicine for its biological activities since ancient times. Leaves of *Vitis vinifera* (grape) is abundant in natural resources as a novel potential antioxidant and has great commercial value.[3] Leaves have astringent and haemostatic properties. Due to these properties leaves are used in the management of varicose veins, diarrhoea, hemorrhage, hemorrhoids, inflammatory disorder, pain, hepatitis, and free radical related diseases and externally for centuries in Anatolia to heal wounds and drain furuncles.[4-5] Leaves juice has been recommended as an antiseptic for eye wash.[6] Leaves are used in the formulation of dietary antioxidant supplements and consumed in traditional foods (Dolmathes) and used for diarrhoea, vomiting and varicose treatment.[7-8]

Vitis vinifera leaves chemical investigations showed the presence of tannins, carotenoids, terpenes, organic acids (malic, oxalic, fumaric, ascorbic, citric, and tartaric acid), phenolic acids, anthocyanins, lipid, enzymes, and reducing or non-reducing sugars.[9-10] According to literature, therapeutic properties of plants were acknowledged due to presence of phenolic compounds. So, phenolic compounds have received considerable attention due to their pharmacological effects including antioxidant, antihyperglycemic and antidyslipidemic activities.[11]

Grape seed, fruit and their extract are already used as functional food or medicinal agent. So, the objective of the present study was to explore the scientific basis for the utility of extracts, qualitative and quantitative analysis of chemical constituent's, and antihyperglycemic activity of *grape* leaves.

2. MATERIALS AND METHODS

2.1. Chemical material

All the chemicals used were of standard analytical grade, purchased from Sigma-Aldrich and Nice chemicals.

2.2. Plant material and preparation of extract

The fresh leaves of *Vitis vinifera* were collected on April 2013 from the Tau Devilal National herbal park, Khizrabad, Haryana, India and authenticated by Dr. Shiddamallayya N., National Ayurveda Dietetics Research Institute, Banglore, India (specimen number RRCBI-MUS-125) (Herbarium in Fig.1). The leaves of the plant were washed and rinsed with tap water and shade dried. The dried leaves were subjected to extraction using methanol (absolute) and water in soxhlet apparatus and resultant filtrate was concentrated under reduced pressure by rotary evaporator. A semisolid paste obtained by this process was stored in refrigerator throughout the study. The extractive value of *Vitis vinifera* methanolic extract (VVME) and *Vitis vinifera* aqueous extract (VVAE) was found as 8.48% and 11.2% w/w respectively.

2.3. Qualitative and Quantitative estimation of Phytoconstituents

The preliminary qualitative screening of extracts was carried out by employing various phytochemical tests. Furthermore, quantitative estimation of different phytoconstituents were carried with UV spectrophotometer using and compared with standards *i.e* for total alkaloids

(standard: atropine), total saponins (standard: diosgenin), total steroids (standard: cycloartenol), total tannin (standard: rutin), total phenolics (standard: gallic acid), total flavonoids (standard: rutin) and total terpenoids was estimated with simple extractive method *i.e* 100 g plant powder were taken separately and soaked in alcohol for 24 hours. Then filtered, the filtrate was extracted with petroleum ether; the ether extract was treated as total terpenoids.[12]

2.4. Animals

Wistar rats (both sex, weight 220-250 g) were used in the experimental protocol and duly approved by Institutional Animal Ethics Committee (MMCP/IAEC/13/36). Animals were kept in animal house of MM College of Pharmacy, Ambala, India as per the guidelines of committee for the purpose of control and supervision of experiments on animals (CPCSEA).

2.5. Induction of diabetes with 10% glucose solution (OGTT method, acute study)

In OGTT method, rats were divided into different groups (n = 6) and fasted overnight and 10% glucose solution per oral was administrated 30 min prior to different doses of extracts i.e. 100, 200 and 400 mg/kg and standard drug metformin (400 mg/kg). Blood samples were collected at different intervals *i.e* 30, 60 and 120 min and glucose level in serum was measured immediately by GOD/POD method using Erba diagnostics kits.[13]

2.6. Induction of diabetes with Alloxan (sub acute study)

Whereas, in alloxan induced diabetes method, hyperglycemia was induced by single intra-peritoneal injection of alloxan (150 mg/kg). Alloxan was freshly prepared by dissolving 150 mg of alloxan in 1 ml of normal saline solution. To prevent the hypoglycaemic shock, 5% glucose was given orally before induction of diabetes in rats. Animals with blood glucose of >200 mg/dl, were included in study. Animals were divided into different groups consisting of six rats each. Metformin was used as standard drug (400 mg/kg).The blood samples withdrawn on 0^{th}, 3^{rd} and 7^{th} day from the retro orbital plexus and serum was separated by centrifugation at a speed of 3000 rpm for 10 minutes. The serum was collected and blood glucose (GOD/POD method), triglycerides, total cholesterol (CHOD-PAP method), HDL (CHOD-PAP Method) with Erba Diagnostics kits was measured and LDL and VLDL are calculated using the formula given below.[14]

$$\text{LDL} = \text{total cholesterol} - \text{total HDL cholesterol} - \left(\frac{\text{triglyceride}}{5}\right)$$

$$\text{VLDL} = \frac{\text{triglyceride}}{5}$$

2.7. Induction of diabetes with Streptozotocin (chronic study)

After overnight fasting, diabetes was induced by injection (i.p.) of streptozotocin (STZ) dissolved in 0.1 M cold sodium citrate buffer, pH 4.5, at a dose of 55 mg/kg. The control rats received vehicle alone the animals were allowed to drink 5% glucose solution overnight to overcome the drug induced hypoglycemia. After 1 week time for the development of diabetes, the rats with moderate diabetes having glycosuria and hypoglycemia (blood glucose range of above 250 mg/dl) were considered as diabetic rats and used for the experiment.

The rats were divided into groups of 6 animals in each group. The standard drug used is glibenclamide (2.5 mg/kg). The plant extracts and standard drug were suspended in 0.9% NaCl in warm water as vehicle solution and administered orally for 28 days.

The fasting blood glucose was measured on 0, 14 and 28 days by GOD-POD estimation kit. After 28 days treatment, the rats were fasted for 16 h. The animals were sacrificed by cervical decapitation and blood collected for estimation of total hemoglobin and HbA1C. A portion of liver tissue was dissected out, washed with ice cold saline immediately and kept at 4^{o}C. The liver tissue was homogenized in 0.1M tris-HCl (pH 7.4) and supernatant was quantified for total cholesterol and triglyceride using kits. A portion of pancreatic tissue was dissected out and fixed in 10% formalin solution and histopathological studies were carried out.[15-16]

2.8. Statistical analysis

Statistical analysis was performed using Dunnett's Multiple Comparisons Test. Values are expressed as mean ± SEM and $p<0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. Qualitative and Quantitative phytochemical screening

The preliminary qualitative screening of VVME and VVAE revealed the presence of flavonoids, phenolic, tannins, steroids, saponins and terpenoids. The amount of total tannins was found to be present in maximum in VVME whereas the other phytoconstituents were found to be present in the following order: total tannin > total flavonoids > total phenolic > total terpenoids > total steroids > total saponin > total alkaloid (Table 1).

Grapes are utilised and grape skins and seeds produced in large quantities by the winemaking industry are increasingly used to obtain functional food ingredients.[17-18] Grapes are the better source of antioxidative constituents than skins of grape/wine byproducts. Functional ingredients of grape seed include several flavonoids with a phenolic nature such as monomeric flavanols, dimeric, trimeric and polymeric procyanidins, and phenolic acids.[2-3] Similarly, in the present study, quantitative estimation of extracts showed the presence of total phenolic, tannin and flavonoids content in higher amount as compare to other ingredients. Methanolic extract was found to possess more amounts of phytoconstituents than aqueous extract.

3.2. Effect of VVME and VVAE on glucose level in OGTT

The basal values of fasting blood glucose level were almost same and statistically no significant difference was observed while including the animals for experimentation. Fasting blood glucose level was measured at 0, 30, 60 and 120 min. Fasting blood glucose level of control group was found to be 74.0 to 80.9 mg/dl whereas significant increase in fasting blood glucose level was observed after administration of 10% glucose in rats i.e. 151.2 mg/dl as compared to control group. Different doses of VVME and VVAE (100, 200 and 400 mg/kg) were administered 30 min before the induction of diabetes and significant reduction in blood glucose level was observed in the treated diabetic rats. However, hypoglycemia was not observed after administration of VVME and VVAE since as per CPCSEA normal blood glucose range of wistar rat is 50-135 mg/dl. Interestingly, 200 and 400 mg/kg of VVME produced significant reduction in blood glucose at 30, 60 and 120 min (Fig.2). Similarly, VVAE at dose 400 mg/kg has shown significant reduction in blood glucose level compared to diabetic control at 60 and 120 min

(Fig.3). In the OGTT, the decrease in blood glucose level was found to be initiated after 30 min and maintained for 2 h. This indicates that it takes about 30 min for the active ingredients or their metabolites present in the extract to enter into the circulation and reach target tissues to bring about antidiabetic effect and duration was found to be 2 h. The levels of serum lipid were found to be non-significant in treated groups as compared to diabetic control in OGTT after 120 min.

3.3. Alloxan induced diabetes

3.3.1. Effect of VVME and VVAE on fasting blood glucose level in alloxan induced diabetes

Administration of Alloxan significantly elevated the serum glucose level in diabetic rats as compared to control group. In alloxan induced diabetic rats serum glucose level was found to be increased on 3rd and 7th day. Whereas, administration of different doses (100, 200 and 400 mg/kg) of extracts significantly attenuated the serum glucose level at 3rd and 7th day from 210-290 to near normal range (120 mg/dl). VVME 400 mg/kg produced maximum decrease in glucose level (124.6 mg/dl) on 7th day. Metformin (400 mg/kg) also significantly attenuated the serum glucose level at 3rd day and 7th day (p<0.001) as compared to alloxan control (Fig.4).

3.3.2. Effect of VVME and VVAE on serum lipid level in alloxan induced diabetes

A significant increase was found in TG, LDL and VLDL level in diabetic control group as compared to control group. The administration of VVME, VVAE and metformin for 7 days significantly attenuated TG, LDL and VLDL. Reduction in TG level was found to be maximum significant at dose 400 mg/kg of VVME. Moreover, in LDL level major attenuation was observed at dose 200 and 400 mg/kg of VVME as compared to diabetic control. Similarly, significant attenuation was observed in LDL level at dose 400 mg/kg of VVAE as compared to diabetic control. Moreover, the VLDL level was significantly attenuated at dose 200 and 400 mg/kg of VVME as compared to diabetic control (Fig.5).

HDL level was found to be significantly reduced in diabetic control group as compared to control group. The significant increase in HDL level was found to be at 200 and 400 mg/kg of VVME and 400 mg/kg of VVAE after 7 days of intervention as compared to diabetic control group. The maximum significant increase in HDL level was found to be at 400 mg/kg of VVME (Fig.6).

The induction of diabetes by alloxan (150 mg/kg *i.p*) and 10% glucose (orally) are well known model. Alloxan, a β-cytotoxin, induces "chemical Diabetes" in a wide variety of animal species including rats by damaging the insulin secreting beta cells.[19] The increased serum glucose level may be due to the partial damage of the pancreatic beta cells. Alloxan is relatively toxic to insulin producing pancreatic β-cells because it preferentially accumulates in β-cells through uptake via the GLUT-2 glucose transporter. This cytotoxic action is mediated by dialuric acid, a reduction product of alloxan. These radicals undergo dismutation to H_2O_2. The action of ROS with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of β-cells, thereby decreasing the secretion of insulin, which in turn increases the blood glucose level.[20] The elevated blood glucose level in the diabetic animals was found to be more than 200 mg/dl, which resembles both type-II diabetes (150 to about 250 mg/dl) with partially functional pancreas as well type-I (above 280 mg/dl) with considerable amount of pancreas damaged.[21] Similar results shows that the administration of alloxan significantly increases the level of

glucose when compared to control, which might account for the cytotoxic effect of alloxan on β-cells.[22] As well as in OGTT model, attenuation in blood glucose level was observed after 30 min. The decline reached at its maximum at 2 h as compared to diabetic control. These results indicate the inhibition of glucose absorption by extracts. It is well known that flavonoids, triterpenoids and phenolics are known to be bioactive antidiabetic principles. Flavonoids also regenerate damaged β cells in the alloxan induced diabetic rats and phenolics found to be effective as antihyperglycemic agents.[23] On the other hand, treatment of Methanolic and Aqueous extracts (100, 200 and 400 mg/kg b.w) for 7 days; significantly attenuated the serum glucose level. Flavonoids also have a role to play in the treatment of diabetes and protect against hyperglycemic and alloxan-induced oxidative stress in experimental animal models.[24]

3.4. Streptozotocin induced diabetes

3.4.1. Effect on the Body weight in Streptozotocin induced diabetes

The antidiabetic effect of extracts were evaluated via streptozotocin induce diabetes in rats for 28 days study. The weight of rats was measured at every week i.e. on 0^{th}, 7^{th}, 14^{th}, 21^{st} and 28^{th} day. There was a significant increase in body weight of normal control as compared to streptozotocin control animals in which body weight was found to be significantly decreased. All animals treated with streptozotocin in diabetic control group showed a significant loss in body weight (g) (from 158.8 g to 136.5 g) which was persistently observed till the end of the study period. Whereas, in 400 mg/kg of VVME and VVAE have significant change in their body weight after 28 days treatment (Table 2). The body weight of STZ-induced diabetic rats were reduced and also recovered after antihyperglycemic treatment. The enhancement of body weight in STZ-induced diabetic treated rats because of increases glucose metabolism.

3.4.2. Effect on the Glucose levels in Streptozotocin induced diabetes

Streptozotocin is a broad spectrum antibiotic extracted from *streptomyces acromogenes*. Like alloxan, streptozotocin causes hyperglycaemia mainly by its direct cytotoxic action on the pancreatic beta cells. In streptozotocin, nitrosourea moiety is responsible for beta cell toxicity, while deoxyglucose moiety facilitates transport across the cell membrane. Like alloxan, the involvement of free radicals generation and resulting alteration of endogenous scavengers of these reactive species have been reported in streptozotocin induced diabetes.[25]

There was found a persistent increase in blood sugar level of streptozotocin induced diabetic control group i.e. from 268.8 to 317.9 mg/dl. Different doses of VVME and VVAE produced significant attenuation in elevated blood glucose level. Moreover, 400 mg/kg of VVME group produced significant attenuation in elevated blood glucose level i.e from 277.3 to 165.5 mg/dl. Similar results were observed with different doses of VVME and VVAE except VVAE 100 mg/kg dose, where the attenuation in blood glucose level was statistically significant (Fig.7).

The treatment of medicinal plant extract to the STZ-induced diabetic rats, that activated the beta cells and granulation return to normal, like to be insulinogenic effect.[26] The glibenclamide is a standard antidiabetic drug, used to compare the antihyperglycemic property in experimental rats. Glibenclamide have been involved in stimulating insulin secretion from pancreatic beta cells principally by inhibiting ATP sensitive KATP channels in the plasma membrane.[27] Courtois et al., have reported that glibenclamide treated STZ-induced diabetic rats showed decrease in blood

glucose level. The previous reports are consistent with our present findings.[28] The decreased level of blood glucose was observed in present study, which indicates that extracts stimulates insulin secretion from the remnant beta cells or regenerated beta cells. The mechanism of the antidiabetic activity of extracts may be involved by increasing either the pancreatic secretion of insulin from the remnant Q cells of the islets of Langerhans. Some plants have antidiabetic activity through insulin releasing stimulatory effects.[29]

3.4.3. Effect on the different lipid levels in Streptozotocin induced diabetes

Lipid plays an important role in the pathogenesis of complications associated with diabetes mellitus. The elevated level of serum cholesterol and reduced level of serum HDL cholesterol in diabetic condition, poses to be a risk of factor for developing microvascular complication leading to atherosclerosis and further cardiovascular diseases like coronary heart disease.[30] The abnormal high concentration of serum lipid in diabetic mainly due to increased mobilization of free fatty acids from peripheral fat depots, and insulin deficiency or insulin resistance may be responsible for dyslipidemia.[17] Whereas VVME and VVAE treated group showed significant improvement in the lipid profile comparable to diabetic control group. Interestingly, 200 and 400 mg/kg of Methanolic extract produced significant attenuation in TG and LDL as compared to diabetic control group. In various studies diabetic rats were found to be possessing high lipid level [31] and similar results were found in our study. The dyslipidemic conditions were also improved in both Methanolic and Aqueous extract dose groups as compared to both normal control and diabetic control.

In streptozotocin control group, the level of serum TC, TG, LDL and VLDL cholesterol was significantly increased, whereas the level of HDL-cholesterol was significantly reduced as compared to normal control group. The administration of VVME and VVAE 400 mg/kg significantly attenuated the level of serum total cholesterol and VLDL level after 28 days study. The level of TG was found to be statistically significant in both VVME and VVAE at 400 mg/kg dose groups (Fig.8). Similarly, the level of serum HDL-cholesterol was found to be significant in both VVME and VVAE 400 mg/kg dose groups (Fig.9).

3.4.4. Effect on the Lipid levels in liver homogenates in Streptozotocin induced diabetes

The cholesterol and triglyceride level were found statistically significantly in VVME 400 mg/kg groups as compared to streptozotocin control. The cholesterol level in VVME 200 mg/kg dose group was also found statistically significant (Fig.10).

The liver is the principal organ occupied with xenobiotics metabolism, such as medicinal plant extracts and also a target tissue where possible toxicity effect of same is first expressed. Therefore, in present study levels of total cholesterol and total triglyceride were investigated in liver homogenates. Although products of lipid digestion are emptied directly into blood via lymph as chylomicrons, chylomicrons travel through the blood stream to supply fatty acids to needed tissues, and their remnants are removed from blood by the liver for recycling. The liver also recovers cholesterol from bile, synthesize more cholesterol and triacylglycerol from excess acetyl units of diets and process them into transport forms – lipoproteins.[32] Moreover, many genetic and acquired disorders like diabetes, atherosclerosis etc may lead to deposits of lipids in vital organs such as liver and kidney, resulting in their impaired function.[33] Therefore, assayed the most common lipids total cholesterol and triglyceride usually implicated in diabetes in the

hepatocytes of diabetic and non-diabetic rats which received extract treatments. The result showed significant decrease in cholesterol and triglyceride levels.

3.4.5. Effect on the Hemoglobin and Hb glycated levels in Streptozotocin induced diabetes

After 28 days, Hb level was significantly reduced in streptozotocin control as compared to normal control. However, a significant increase in Hb concentration was observed in all treated groups of VVME and VVAE except VVAE 100 mg/kg, when compared to streptozotocin control (Fig.11). After 28 days of treatment, the mean HbA1c level was significantly decreased in VVME 200 mg/kg, VVME 400 mg/kg and VVAE 400 mg/kg groups as compared to streptozotocin control. The excess of glucose is present in the blood during diabetes, which react with hemoglobin and form glycosylated hemoglobin. The various proteins including hemoglobin, albumin, collagen and low density lipoprotein (LDL)/crystalline proteins undergo nonenzymatic glycation in diabetes. The hemoglobin level was decreased in diabetic rats that may increase the formation of glycosylated hemoglobin. Glycosylated hemoglobin was found to be increased in diabetic mellitus and the amount of increase is directly proportional to that of fasting blood glucose level. The significant decrease in glycosylated hemoglobin indicated that the efficiency of extracts in glycemic control.[34]

3.5. Histopathology of Pancreas in Streptozotocin induced diabetes

It has been noted that abnormal lipid results, often lead to disturb normal architecture in pancreas or other tissues. So, ahead to histopathology of pancreas clearly indicate in restoration of near normal architecture pancreatic islets of langerhans and hepatocytes in extracts and glbenclamide. Histological section of pancreas of normal control rat showed pancreatic lobules separated by connective tissue septa. The pancreatic lobules consisted largely of the exocrine acini and their intralobular ducts. Most of the lobules showed small, round, light-staining islets of langerhans. The center of islet cells consisted of aggregates of small β-cells (70%) having basophilic granules, while the periphery comprised of large α-cells (25%) having eosinophilic granules. Intervening these cells were seen thin walled capillaries. Diabetic control (streptozotocin) rat pancreas section showed pancreatic lobules separated by connective tissue septa. Some of the lobules showed small, round, light-staining islets of langerhans. The center of islet cells consisted of quantitative decrease in β-cells (40%) having basophilic granules, while the periphery comprises of large α-cells (55%) having eosinophilic granules. Scattered lymphocytes within the islet cells were also seen. Glibenclamide rat pancreas section showed pancreatic lobules separated by connective tissue septa. Most of the lobules show large areas of light-staining islets of langerhans. The center of islet cells consisted of mild quantitative increase in β-cells (75%) having basophilic granules, while the periphery comprised of α-cells having eosinophilic granules. VVAE 400 mg/kg section showed pancreatic lobules separated by thin connective tissue septa. Most of the lobules showed small areas of light-staining islets of Langerhans. The center of islet cells consisted of quantitative decrease in β-cells (70%), while the periphery comprised of α-cells (50%) having eosinophilic granules. VVME 400 mg/kg section showed pancreatic lobules separated by thin connective tissue septa. Most of the lobules showed large areas of light-staining islets of langerhans. The center of islet cells consisted of quantitative increase in β-cells (45%) having basophilic granules, while the periphery comprised of α-cells (20%) having eosinophilic granules.

4. CONCLUSION

The study reveals the antihyperglycemic and antidyslipidemic activity of VVME and VVAE in rats. Also, shows these extract might be useful both in type-II and type-I diabetes, irrespective of whether the pancreas is partly functional or almost totally destroyed. However the precise mechanism by which *Vitis vinifera* reduced blood glucose level in diabetic rats requires further study. Therefore, future research and clinical trials in this area may lead to the use of grape leaves as a new type of therapeutic agent in treatment of diabetes. As well as, further pharmacological studies of isolated compounds may provide a new therapeutic agent.

With previous studies, it is clear that food and pharmaceutical companies are committed to the study and development of functional foods and other nutraceuticals. They also have demonstrated that a sound scientific base for the efficacy of these products combined with good marketing leads to acceptance by consumers for various types of products. From the present study it is clear that, grape leaves can be good functional food option for both food and pharmaceutical companies.

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6. AUTHOR DISCLOSURE STATEMENT

The author declare that there is no conflict of interest

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Table 1. Quantitative estimation of total phytoconstituents content present in *Vitis vinifera* leaves extract.

Total phytoconstituents content	***Vitis vinifera* extract**		**Standard equivalent** mg/g
	VVME	**VVAE**	
Total Tannin content	36.37±0.51	33.27±0.32	Rutin
Total Flavonoid content	29.45±0.31	34.10±0.26	Rutin
Total Phenolic content	22.27±1.69	27.43±2.42	Gallic acid
Total Terpenoids content	5.97±1.67	5.47±1.21	-----
Total Steroid content	4.89±2.59	3.31±1.36	Cycloartenol
Total Saponin content	0.77±0.61	------	Diosgenin
Total Alkaloid content	------	------	Atropine

Table 2. Effect of VVME and VVAE on % changes in body weight in streptozotocin induced diabetes.

Groups/treatment	% change in body weight
Normal Control	11.3%
Streptozotocin control	-14.1%
Glibenclamide	15.95%
VVME 100mg/kg	-2.51%
VVME 200mg/kg	-0.63%
VVME 400mg/kg	5.75%
VVAE 100mg/kg	-0.62%
VVAE 200 mg/kg	1.24%
VVAE 400 mg/kg	7.05%

Figure 1. *Vitis vinifera* L. leaves

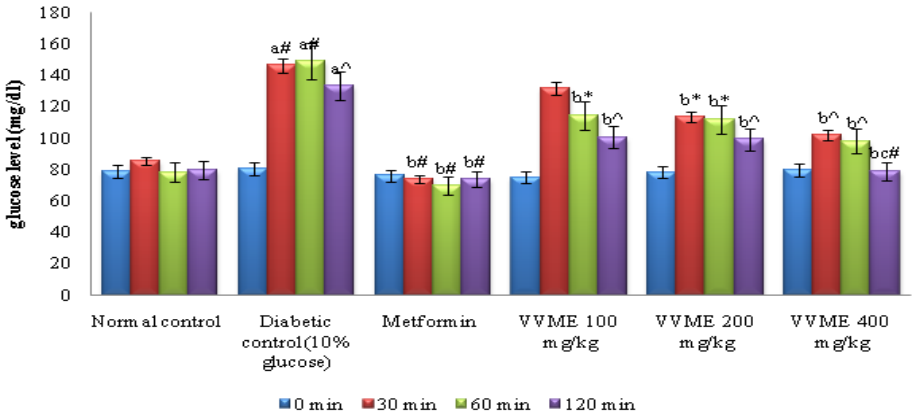


Figure 2. Effect of VVME on Fasting Blood Glucose Level in OGTT. Values are represented as mean±SEM, n=6. Statistically analysis was done with Dunnett's Multiple Comparison Test and $p<0.05$ was considered to be statistically significant; b = *vs* diabetic control; c = *vs* 100 mg/kg dose. $p<0.05$= *; $p<0.01$= ^ and $p<0.001$ = #.

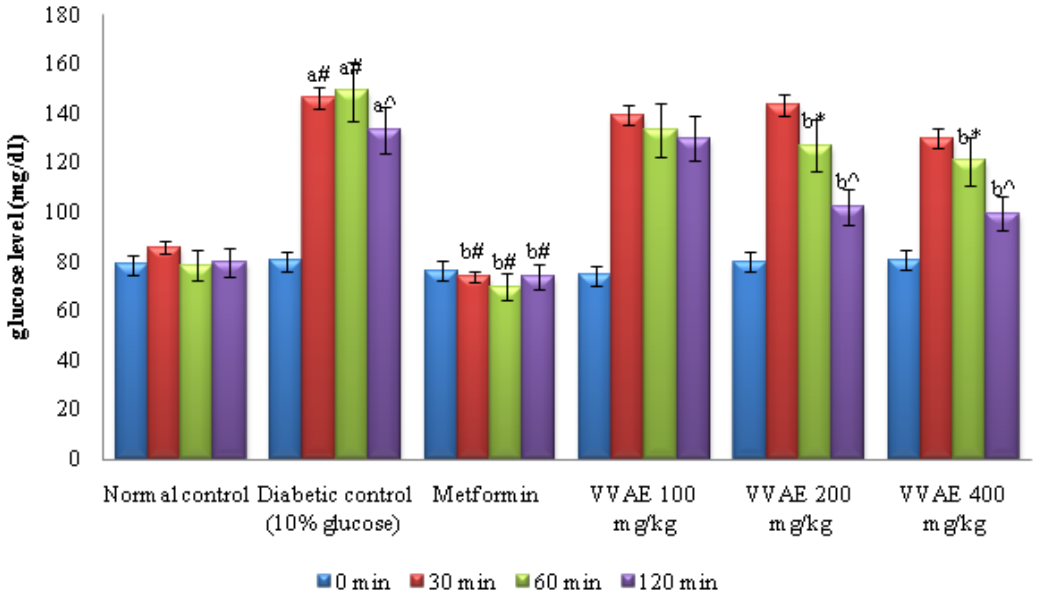


Figure 3. Effect of VVAE on Fasting Blood Glucose Level in OGTT. Values are represented as mean±SEM, n=6. Statistically analysis was done with Dunnett's Multiple Comparison Test and $p<0.05$ was considered to be statistically significant; b = *vs* diabetic control. $p<0.05$= *; $p<0.01$= ^ and $p<0.001$ = #.

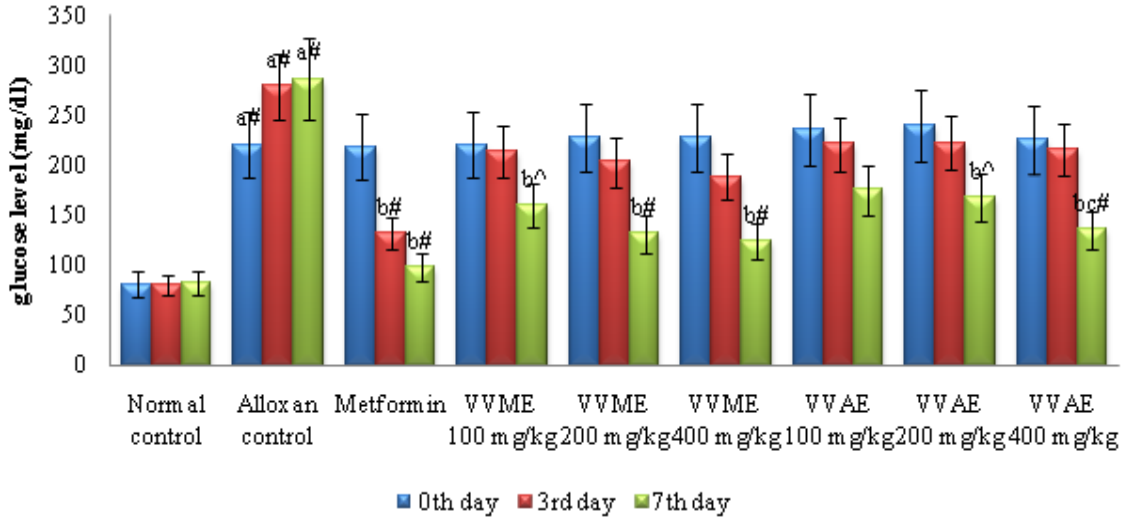


Figure 4. Effect of VVME and VVAE on Fasting Blood Glucose Level in Alloxan induced Diabetes. Values are represented as mean±SEM, n=6. Statistically analysis was done with Dunnett's Multiple Comparison Test and $p<0.05$ was considered to be statistically significant; b = *vs* alloxan control; c = *vs* 100 mg/kg dose. $p<0.05$= *; $p<0.01$= ^ and $p<0.001$ = #.

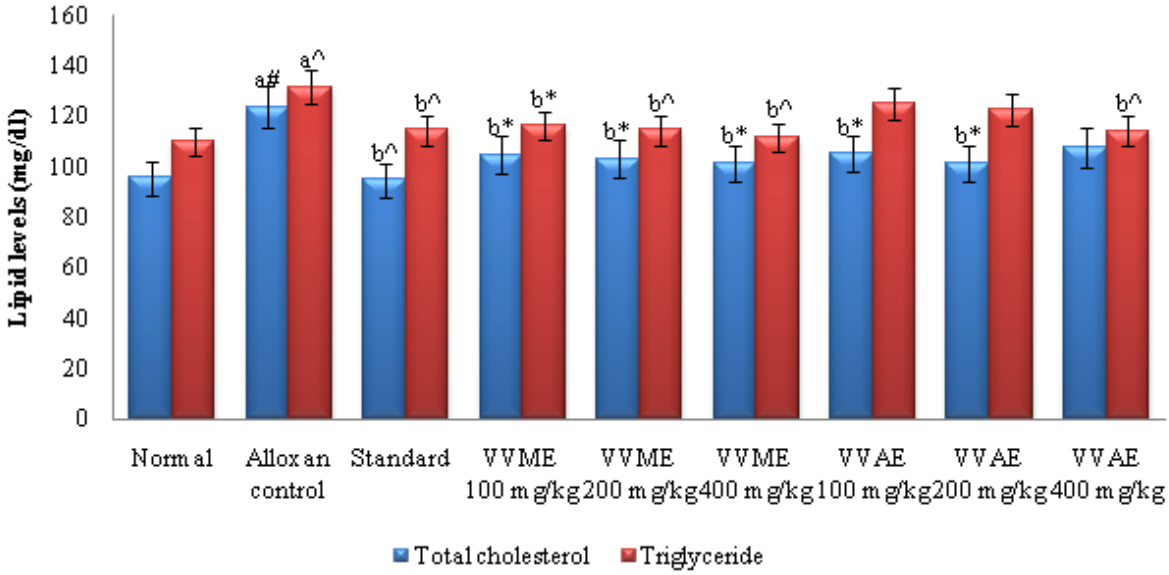


Figure 5. Effect of VVME and VVAE on serum total cholesterol and triglyceride levels in alloxan induced diabetes. Values are represented as mean±SEM, n=6. Statistically analysis was done with Dunnett's Multiple Comparison Test and $p<0.05$ was considered to be statistically significant; b = *vs* alloxan control. $p<0.05$= *; $p<0.01$= ^ and $p<0.001$ = #.

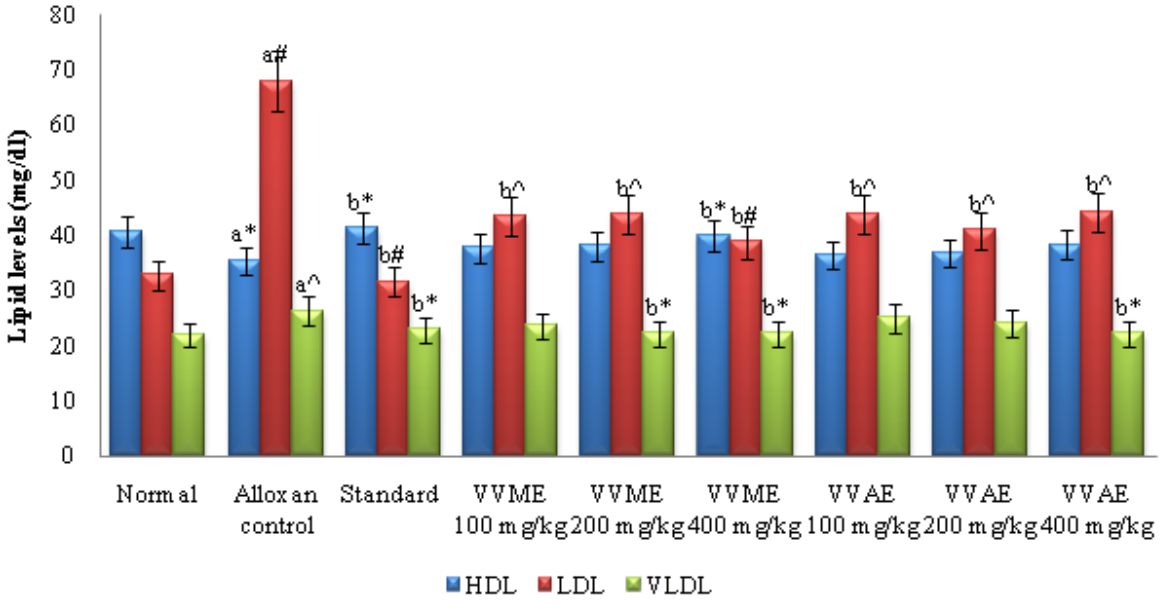


Figure 6. Effect of VVME and VVAE on serum lipids levels in alloxan induced diabetes. Values are represented as mean±SEM, n=6. Statistically analysis was done with Dunnett's Multiple Comparison Test and $p<0.05$ was considered to be statistically significant; b = *vs* alloxan control. $p<0.05$= *; $p<0.01$= ^ and $p<0.001$ = #.

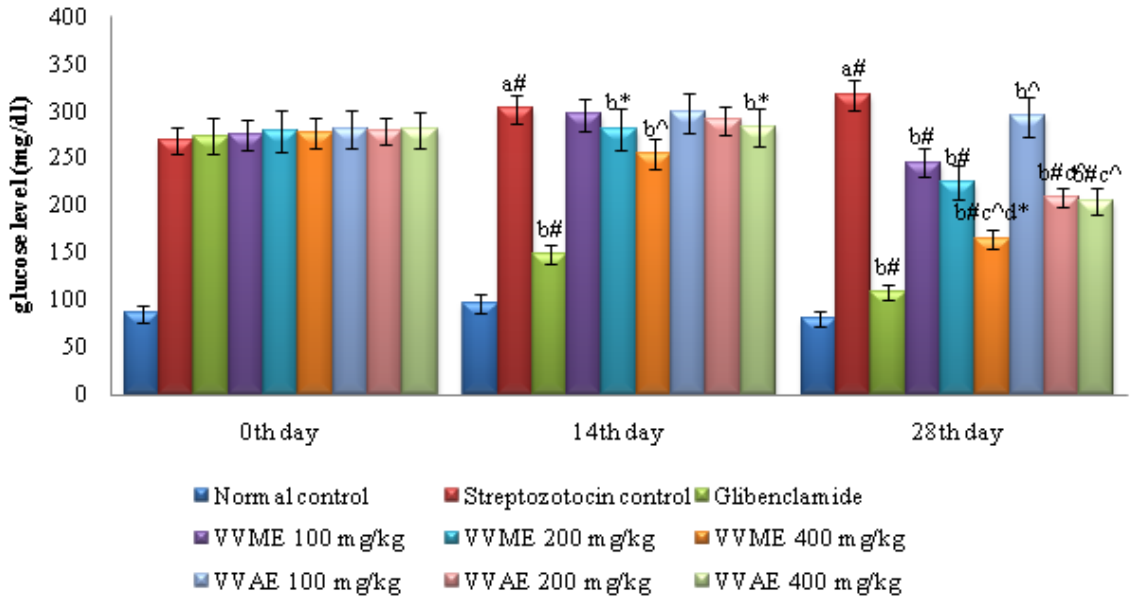


Figure 7. Effect of VVME and VVAE on glucose level in streptozotocin induced diabetes. Values are represented as mean±SEM, n=6. Statistically analysis was done with Dunnett's Multiple Comparison Test and $p<0.05$ was considered to be statistically significant; b = *vs* streptozotocin control; c = *vs* 100 mg/kg dose; d = *vs* 200 mg/kg dose. $p<0.05$= *; $p<0.01$= ^ and $p<0.001$ = #.

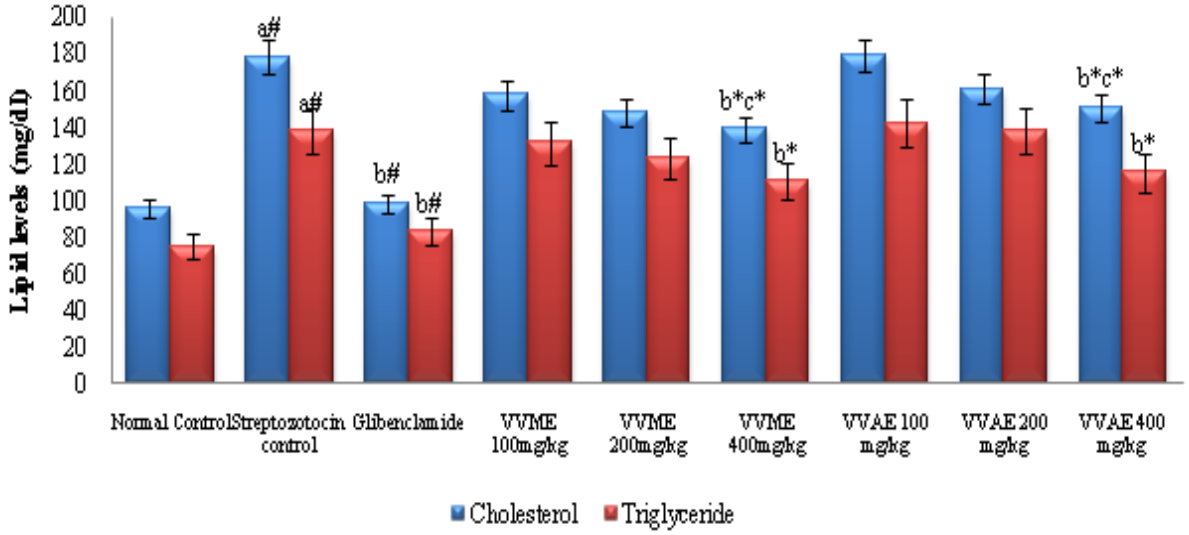


Figure 8. Effect of VVME and VVAE on lipid levels in streptozotocin induced diabetes. Values are represented as mean±SEM, n=6. Statistically analysis was done with Dunnett's Multiple Comparison Test and $p<0.05$ was considered to be statistically significant; b = *vs* streptozotocin control; c = *vs* 100 mg/kg dose. $p<0.05$= *; $p<0.01$= ^ and $p<0.001$ = #.

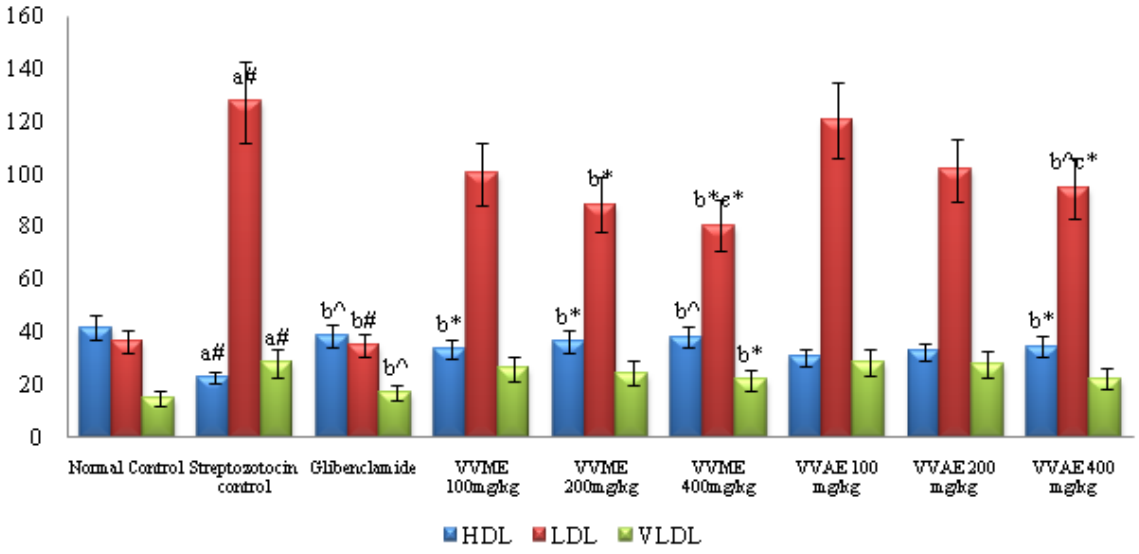


Figure 9. Effect of VVME and VVAE on lipid levels in streptozotocin induced diabetes. Values are represented as mean±SEM, n=6. Statistically analysis was done with Dunnett's Multiple Comparison Test and $p<0.05$ was considered to be statistically significant; b = *vs* streptozotocin control; c = *vs* 100 mg/kg dose. $p<0.05$= *; $p<0.01$= ^ and $p<0.001$ = #.

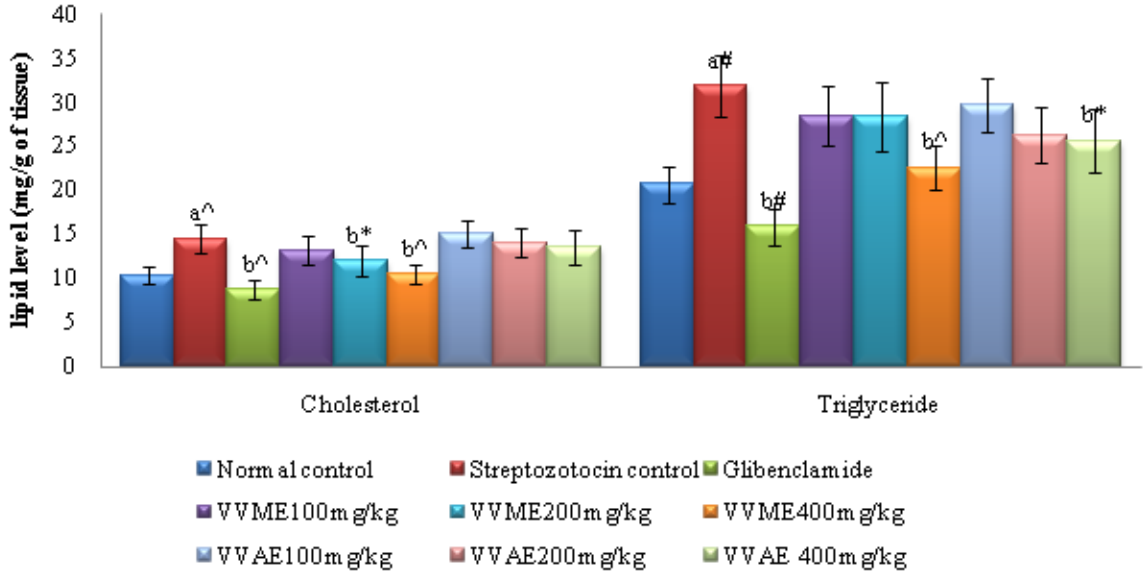


Figure 10. Effect of VVME and VVAE on lipid levels in liver homogenates in streptozotocin induced diabetes. Values are represented as mean±SEM, n=6. Statistically analysis was done with Dunnett's Multiple Comparison Test and $p<0.05$ was considered to be statistically significant; b = *vs* streptozotocin control. $p<0.05$= *; $p<0.01$= ^ and $p<0.001$ = #.

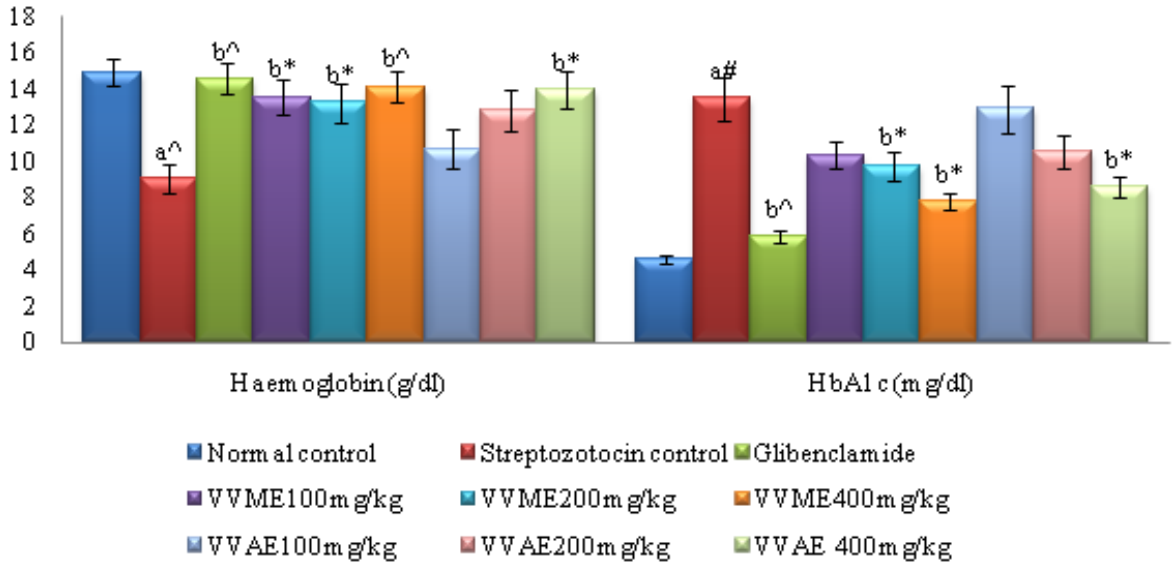
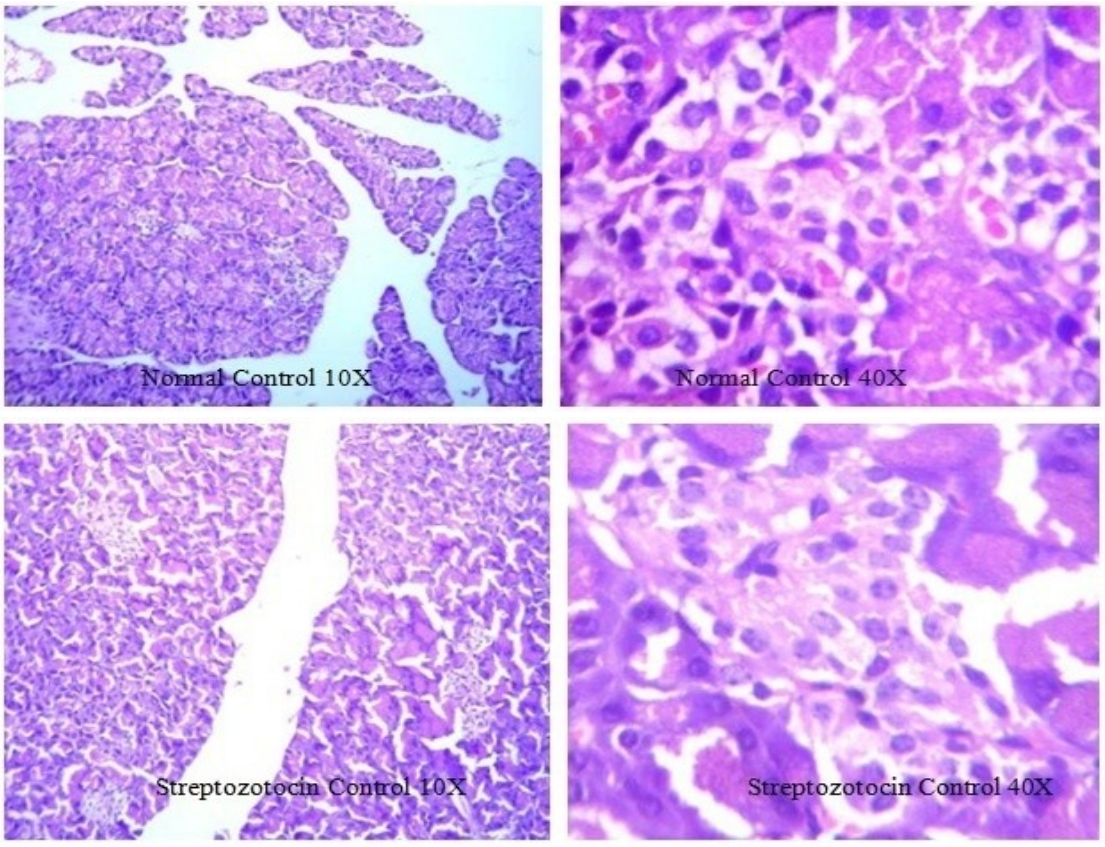


Figure 11. Effect of VVME and VVAE on hemoglobin and hb glycated levels in streptozotocin induced diabetes. Values are represented as mean±SEM, n=6. Statistically analysis was done with Dunnett's Multiple Comparison Test and $p<0.05$ was considered to be statistically significant; b = *vs* streptozotocin control. $p<0.05$= *; $p<0.01$= ^ and $p<0.001$ = #.



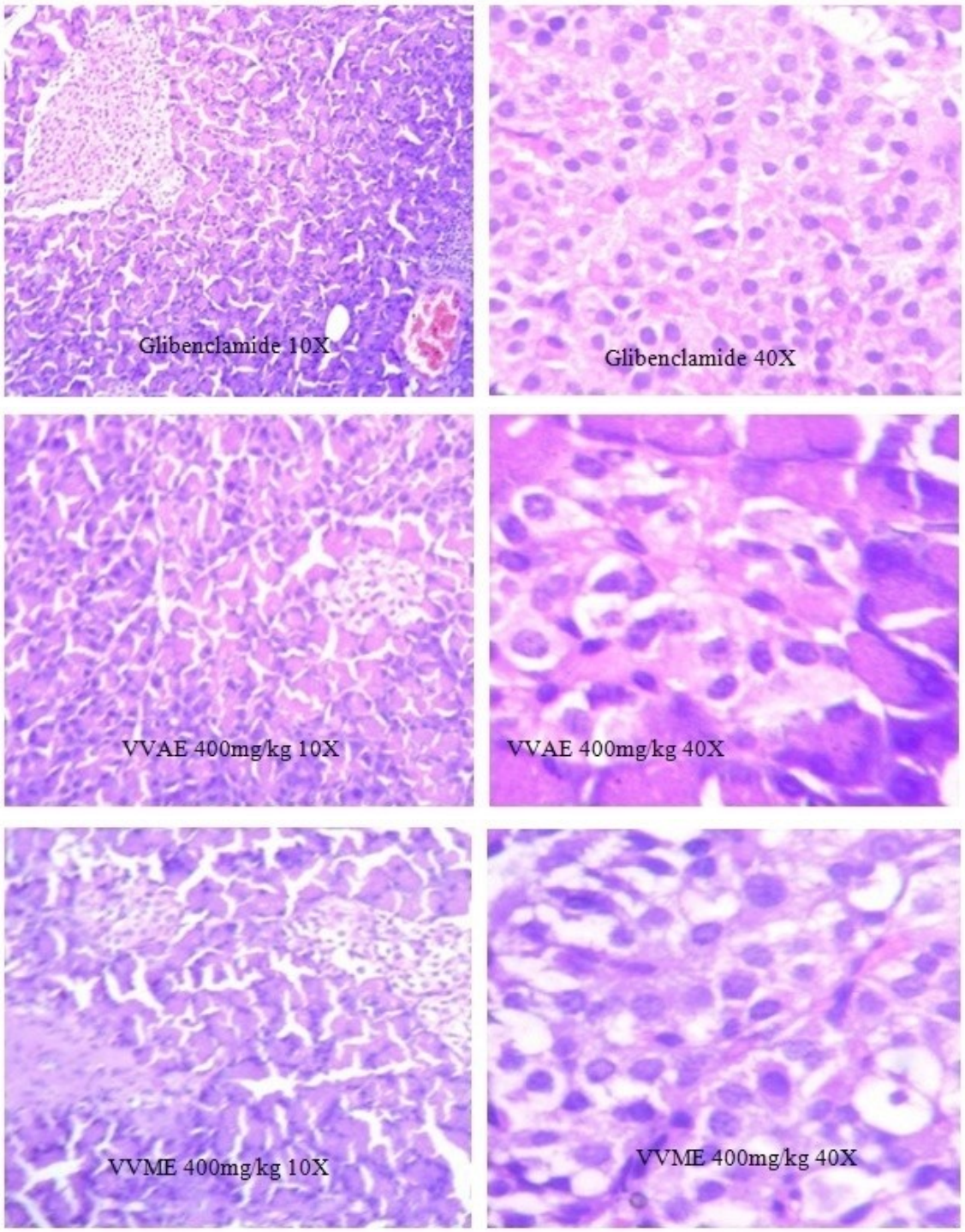


Figure 12. Histopathology images of pancreas in streptozotocin induced diabetes