European Journal of Molecular & Clinical Medicine

ISSN 2515-8260 Volume 08, Issue 03, 2021

Assessment of antidiabetic activity of hydroalcoholic extract of *Volvariella volvacea*

Pushpa P. Gupta[1], Shashikant Chandrakar[1*], Amit Roy[1], Seema Verma[2], Neeta Gupta[3], Ram Kumar Sahu[4]

[1]Columbia Institute of Pharmacy, Mandhar, Raipur (C.G.)-493111

[2]Royal College of Pharmacy, Raipur (C.G.)

[3]Govt. E. Raghvendra Rao P. G. Science College, Bilaspur(C.G.)

[4]Department of Pharmaceutical Science, Assam University (A Central University) Silchar, Assam

Corresponding author: e-mail: shashikant.py@gmail.com

Mobile:-+919926752396

Abstract

Mushrooms (a macrofungus) have high dietary values and are used in cooking for ages. Some of them are also reported to have medicinal properties. Volvariella volvacea (V. Volacea) is an edible mushroom cultivated in East and South East Asia. This provides good nutrition is extremely suitable in human diet. The present work was planned to determine the antidiabetic potential of V. volvacea by exploring the glucose tolerance test and other vital serum profiles in normal as well as STZ-induced diabetic rats. The mushroom was subjected to hydroalcoholic extraction; the extract was divided into two equal portions. One part was dried by lyophilisation (LE) and second part was dried in hot air oven (ODE). The LE and ODE of V. volvacea was studied for antidiabetic activity. The extracts, in a dose dependent manner could significantly decrease the serum glucose level of diabetic rats. The results show that LE of V. volvacea exhibited more antidiabetic activity than ODE. The above result advocate that more study is required to proceed for developing a formulation of this mushroom to utilise its nutraceutical and medicinal properties.

Key words: Mushroom, Lyophilization, serum profile, Nutraceuticals

1 Introduction

Diabetes particularly type-II diabetes usually develops due to increased oxidative stress[1], which is produced due to imbalance between production and scavenging of free radicals . The free radicals are produced due to radiation, enzymatic metabolism of drugs, mitochondrial respiration, or exogenous chemicals. These free radicals injure nucleic acids, proteins and lipids [2]. To overcome this, nutraceuticals and other dietary substances having antioxidant activity are used. From centuries mushrooms are known for their edible as well as medicinal properties. The mushrooms were first used by the Egyptians and Chinese, and Hippocrates in 400 B.C. showed their medicinal value [3]. Till date about 14000 mushrooms have been identified [4], but only 2000 mushrooms are used for edible purpose or as medicinal mushrooms. Edible mushrooms are highly nutritious [5]. They are rich in proteins, vitamins,

minerals, and fibres and they do not contain cholesterol [6]. Many studies have reported mushrooms contain an enormous amount of secondary metabolites having antioxidant activity.

V. volvacea is an edible mushroom species that has high amount of polypeptides, terpenes and steroids [7]. It also contains phenolic phytoconstituents namely tannins, phenolic acids, and flavonoids. Presences of these compounds contribute to high antioxidant capability [8]. Ames *et al.* (1993) reported that antioxidant action of *V. volvacea* safeguards against the risks of chronic angiogenic ailments for instance cardiovascular diseases, cancer, inflammation as well as arthritis [9]. Ram kumar *et al.* (2012) also has shown the presence of appreciable levels of antioxidants. They have also mentioned the presence of glutathione peroxidase, superoxide dismutase, glutathione-S-transferase catalase, peroxidase, and glutathione reductase in dried mushroom and even in mycelial mats [10].

2 Materials and methods

2.1 Collection and identification of mushroom

The spawn of *V. volvacea* was acquired from Mushroom Research Centre, Department of Plant Pathology, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India. It was then cultivated in Institutional Columbia Mushroom Centre, under the expert guidance of scientist from Indira Gandhi Krishi Vishwavidyalaya, Raipur and Chhattisgarh Council of Science and Technology, Raipur, Chhattisgarh, India.

2.2 Preparation of mushroom sample

Both the mushrooms were washed to remove dirt and adhering materials. They were subjected to sun drying to dry the increase in moisture due to washing. Thereafter they were dried under shade till free from moisture. Mushroom species were ground separately to get coarse powder. Thereafter they were stored separately in labelled air tight container for further use.

2.3 Extraction procedure

The prepared sample of mushroom was extracted with different solvent as shown in above figure (Figure no. 1).

2.3.1 Extraction with Petroleum ether

Powdered drug was macerated with petroleum ether for seven days. This solution was kept on orbital shaker for six hours every day to enhance extraction. The resulting petroleum ether extract was filtered, and the extract was dried in water bath. Marc obtained was dried and then it was kept for extraction with other solvents. The petroleum ether extracts of both the mushrooms was kept for further experimental work in refrigerator.

2.3.2 Extraction with hydroalcoholic solvent

The marc was further extracted with hydroalcoholic solution (70:30). For this the mark was immersed in the solution and was macerated for 7 days and during this time it was kept on orbital shaker to enhance the extraction process. Thereafter it was filtered and the marc was discarded [11].

2.3.3 Drying of hydroalcoholic extract

The hydroalcoholic extract was divided into two equal parts. One part was subjected to lyophilisation at -20ºC.The second portion was dried in tray dryer at 50ºC constant temperature and weighed periodically till a constant weight was observed. The lyophilized extract (LE) and the dried extract (ODE) were stored in refrigerator in air tight container for additional experimental work.

2.4 *In vivo* study

The streptozotocin- nicotnamide induced diabetic rats model was used to assess the antidiabetic potential of LE and ODE of *V. volvacea.* Male Wistar rats weighing ranged from 150 to 230 gm were used for the study. Seven groups were made and each group contained six rats. Each group was kept separately in polyacrylic cages (38 x 23 x 10 cm). The rats were maintained under standard laboratory conditions (temperature 25 ± 2ºC) with dark/ light cycle (14 /10 h). They were given with standard dry pellet diet and water ad libitum. The rats were allowed to get accustomed to laboratory conditions for 10 days before beginning the experiment. All testing were approved by the institutional ethical committee and experiments were done as per its guidelines.

2.4.1 Initiation of non-insulin dependent diabetes mellitus (NIDDM)

The rats were fasted for overnight. A single dose of streptozotocin was administered intraperitonally to induce NIDDM, at a dose of 60 mg/kg. This was done 15 minutes after nicotinamide administration (120 mg/kg i.p.). Nicotinamide was dissolved in normal saline and STZ was dissolved in citrate buffer at a pH of 4.5. The glucose level in plasma was measured after 72 hours and then on 7th day. The level of glucose measurement confirmed initiation of hyperglycemia. The entry value of fasting plasma glucose level was >126mg/dl for diagnosis of diabetes. Only those that showed permanent NIIDM were used for the study [12, 13].

2.4.2 Experimental design

The animals were separated into seven groups. The group size was six (n = 6).

Group I- The non diabetic normal control

Group II – The Diabetic control

Group III- In this group rats were given standard drug Glibenclamide (0.5 mg/kg)

Group IV- In this group rats were given LE (200 mg/kg);

Group V- In this group rats were given LE (400 mg/kg); and

Group VI- In this group rats were given ODE (200 mg/kg); and

Group VII - In this group rats were given ODE (400 mg/kg) for 28 days.
On 0, 7th, 14th and 28th days of administering the extract, the fasting glucose levels were determined. During the experimental tenure, weight of rats was measured every day and mean change was calculated.

2.4.3 Estimation of insulin level
After 28th days of treatment of LE and ODE blood samples were withdrawn to determine insulin levels. Serum insulin was calculated using a GLAZYME INSULIN-EIA TEST.

2.4.4 Estimation of biochemical parameters
The rats were sacrificed by cervical dislocation on 28th day for determining various biochemical parameters. The glucose oxidase method was used for the determination of total cholesterol, triglycerides (TGL), high-density lipoprotein (HDL) and low-density lipoprotein (LDL). Triglycerides and cholesterol kit (Minias Globe Diagnostic kit) was used for the analysis of sample by using semi auto-analyzer.
The serum samples from all the groups were also subjected for the study of aspartate aminotransferase (AST), serum alkaline phosphatase (ALP), serum alanine aminotransferase (ALT), lactate dehydrogenase (LDH), urea and creatinine levels with kits purchased from Crest Biosystems (India) [14,15].

2.4.5 Statistical analysis
The results are expressed as mean ± SEM of six independent experiments. Statistical significance between the groups was evaluated first by one-way analysis of variance (ANOVA) and then by Dunet's test. A $P < 0.05$ value was considered as statistically significant.

3 Results and Discussions
Antidiabetic activity of *V. volvacea*
3.1 Effect of LE and ODE of *V. volvacea* on hyperglycemia
The effect of LE and ODE of *V. volvacea* and glibenclamide on serum glucose levels in different groups of rats is presented in Table 1. The blood glucose level of diabetic rat significantly increased compared to normal rats. The rats treated with LE and ODE at two different doses (200 mg/kg and 400 mg/kg) to STZ-induced diabetic rats exhibited significant reduction of blood glucose level. The maximum decrease in blood glucose in rats was observed on 28th day. The blood glucose level of LE at the dose 200 mg/kg and 400 mg/kg group was found to be 99.18±3.57 mg/dl and 73.65±5.92 mg/dl, respectively at the end of study. While ODE treated rats at the dose of 200 mg/kg and 400 mg/kg exhibited 105.32±3.47 mg/dl and 89.71±2.61 mg/dl blood glucose level, respectively.

3.2 Effect of LE and ODE of *V. volvacea* on lipid profile
The LE and ODE of *V. volvacea* (200 mg/kg and 400 mg/kg) considerably decreased the levels of cholesterol, triglycerides and LDL in diabetic rats in comparison to the diabetic

control group (Table 2). Consequently, the HDL significantly increased in groups treated with extracts and standard in comparison to diabetic control group.

3.3 Effect of LE and ODE of *V. volvacea* on body weight

The body weight of rats of diabetic control group decreased drastically on induction of diabetes. The extract and glibenclamide treated rats were found to gain body weight in comparison to diabetic control group as shown in Table 3.

3.4 Effect of LE and ODE of *V. volvacea* on serum insulin levels

The decrease in serum insulin level indicates hyperglycemia in rats. The STZ-induced diabetic rats exhibited maximum decrease in serum insulin levels on 28th day. The administrations of LE, ODE and Glibenclamide to diabetic rats for 28th day resulted in significant increase in serum insulin levels. (Table 4)

3.5 Effect of LE and ODE of *V. volvacea* on liver and renal serum biomarkers

The increased level of AST, ALT, ALP, LDH, urea and Creatinine in serum of diabetic rats indicates abnormal function of liver and kidney. The AST, ALT, ALP, LDH, urea and Creatinine in serum were increased significantly in diabetic control group compared to normal control group. Consequently, administration of LE, ODE and Glibenclamide to diabetic rats resulted in significant decrease in biochemical parameters, representing improvement in liver and kidney function. (Table 5)

Studies for anti-diabetic activity of *V. volvacea* showed that blood glucose level of LE at dose of 200 mg/kg and 400 mg/kg group was found to be 99.18±3.57 mg/dl and 73.65±5.92 mg/dl, respectively at the end of study. While ODE treated rats at the dose of 200 mg/kg and 400 mg/kg exhibited 105.32±3.47 mg/dl and 89.71±2.61 mg/dl blood glucose level, respectively. The levels of cholesterol, triglycerides and LDL in diabetic rats were significantly lowered in LE and ODE of *V. volvacea* (200 mg/kg and 400 mg/kg) treated group compared to diabetic control group. In diabetic control group body weight significantly deceased. When compared to diabetic control group, extract and glibenclamide treated rats were found to gain body weight. Serum insulin levels of rats were decreases in STZ-induced diabetic rats on 28th day where as significant increase in serum insulin level was observed on administrations of LE, ODE and Glibenclamide to diabetic rats. The abnormal function of liver and kidney was determined by increased level of AST, ALT, ALP, LDH, urea and creatinine in serum.

On comparing the normal control group LDH, ALP. AST, ALT, urea and Creatinine in serum increased appreciably in diabetic control group. As a result, administration of Glibenclamide, LE and ODE to diabetic rats resulted in noteworthy decrease in biochemical parameters, show that there was improvement in liver and kidney function.

4 Conclusion

In present study *V. volvacea,* an edible mushroom of Chhattisgarh was selected. The findings of this study showed that Lyophilized extract (LE) of *V. volvacea* significantly reduced blood glucose level in diabetic rats when compared to diabetic control group. Similarly in

determination of lipid profile LE extract of *V. volvacea* showed significant result compared to diabetic control group. In body weight observation, both LE and ODE treated rats showed gain in body weight compared to diabetic control group. Increased insulin level was also found on 28th day in LE, ODE and Glibenclamide treated rats. In diabetic rats levels of LDH, AST, ALT, urea and creatinine were increased appreciably whereas diabetic rats treated with LE, ODE and Glibenclamide a noteworthy decrease in these parameters were observed which signifies proper functioning of liver and kidney. Above result show that *V. volvacea* has significant antidiabetic activity. Further isolation and characterization of phytochemicals responsible for antidiabetic activity has to be done for commercialization of the product.

5 Acknowledgement

The authors would like to pay their sincere attitude towards the management of Columbia Institute of Pharmacy, Raipur for providing the infrastructure and facilities for the completion of this work.

6 Conflict of interest statement

There is no conflict of interest.

7 References

1. Pham-Huy LA, He H, Pham-Huy C. Free Radicals, Antioxidants in Disease and Health. Int J Biomed Sci, 2008; 4(2): 89–96.
2. Kumar V, Khan AA, Tripathi A, Dixit PK, Bajaj UK. Role of oxidative stress in various disease: Relevance of dietary antioxidants. The journal of Phytopharmacology, 2015; 4(2): 126-132.
3. Lo HC, Wasser SP. Medicinal mushrooms for glycemic control in diabetes mellitus: history, current status, future prespective and unsolved problems (Review).Int. J. Med. Mushrooms, 2011; 13(5): 401-26.
4. Hawksworth DL. The magnitude of fungal diversity: the 1.5 million species estimated revisited. Mucological Research, 2001; 105(12) : 1422-1432.
5. Oei P 2005. Small scale mushroom cultivation- oyster, shitake and wood ear mushroom. Digigrafi, Wageningen, The Neterlands.
6. Isikhuemhen OS, Nerud F. Preliminary studies on the ligninolytic enzymes produced by the tropical fungus *Pleurotus tuber-regium*. Antonie van Leeuwenhoek, 1999; 75: 257-260.
7. Shwetha VK, Sudha GM. Ameliorative effect of *Volvariella volvacea* aqueous extract (Bulliard Ex Fries) Singer on gentamicin induced renal damage. International Journal of Pharma and Bio Sciences, 2012; 3(3):105-117.
8. Roy A, Prasad P. Determination of physicochemical, phytochemical and antioxidant activity of *Volvariella volvacea*. American Journal of Pharmtech Research, 2016; 6(2): 641-654.
9. Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants and the degenerative diseases of aging. Proc Nati Acad Sci, 1993; 90(17): 7915-7922.

10. Ramkumar L, Ramanathan T, Johnprabagaran J. Evaluation of nutrients, trace metals and antioxidant activity in *Volvariella volvacea* (Bull. Ex. Fr.) Sing. Emir J Food Agric, 2012; 24(2): 113-119.
11. Harbone JB. 2005. Phytochemical Methods – a guide to modern techniques of plant analysis. Springer, New Delhi.
12. Singh J, Sahu RK, Prasad DN, Jangde R, Gupta R. Evaluation of antidiabetic potential of *Ougeinia oojeinensis* leaves in streptozotocin-induced-diabetic rats. Pharmacologyonline, 2011; 2: 1046-1052.
13. Maurya AK, Tripathi S, Kahrana M, Ahmed Z, Sahu RK. Evaluation of antidiabetic effects of *Ficus racemosa* extracts in diabetic induced rats. Pharmacologyonline, 2011; 3: 907-914.
14. Kumar S, Kumar V, Prakash O. Antidiabetic, hypolipidemic and histopathological analysis of *Dillenia indica* (L.) leaves extract on alloxan induced diabetic rats. Asian Pacific Journal of Tropical Medicine, 2011; 4(5): 347–352.
15. Schmatza R, Perreiraa LB, Stefanelloa N, Mazzantia C, Spanevelloa R, Gutierresa CJ. Effects of resveratrol on biomarkers of oxidative stress and on the activity of delta aminolevulinic acid dehydratase in liver and kidney of streptozotocin-induced diabetic rats. Biochimie, 2012; 94(2):374–383.

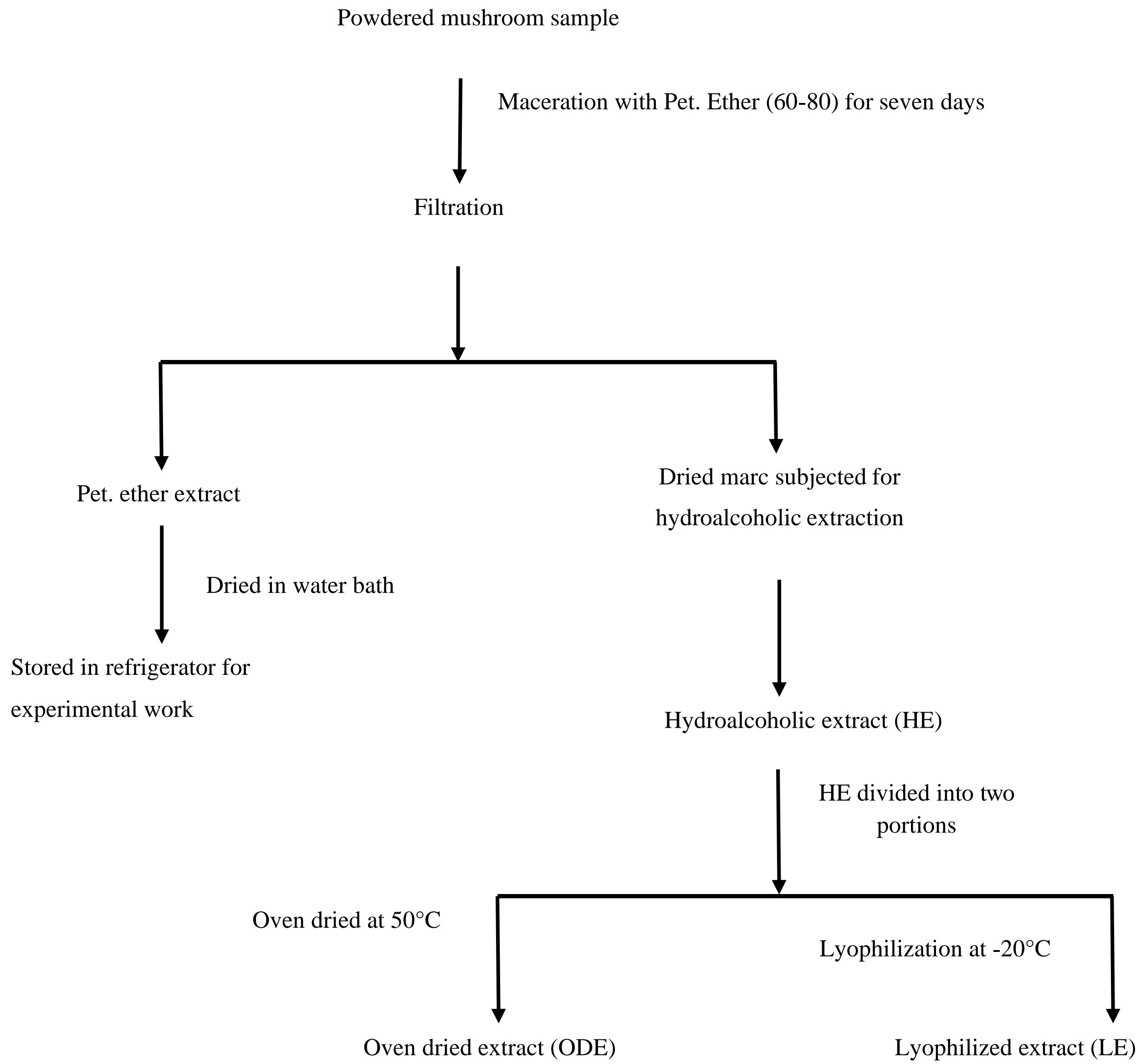

Fig. No. 1 Extraction Scheme

Table 1: Effect of LE and ODE of *V. volvacea* on fasting plasma glucose level in rats

Group	Fasting plasma glucose concentration (mg/dl)			
	Day 0	Day 7th	Day 14th	Day 28th
Normal Control	79.35±3.62	75.24±4.59	77.14±5.67	79.21±3.48
Diabetic control (Streptozotocin)	135.28±4.15a	213.82±5.20a	253.42±4.28a	296.58±5.26 a
Diabetic + Standard Glibenclamide (0.50 mg/kg)	140.63±4.57	112.58±3.82*	91.42±4.81*	74.46±3.58*
Diabetic + LE (200 mg/kg)	137.52±2.85	137.14±5.81*	119.62±4.38*	99.18±3.57*
Diabetic + LE (400 mg/kg)	134.81±6.14	116.43±6.73*	95.35±2.93*	73.65±5.92*
Diabetic + ODE (200 mg/kg)	136.54±3.69	149.32±2.54*	120.14±5.34*	105.32±3.47*
Diabetic + ODE (400 mg/kg)	140.17±4.19	132.59±3.86*	115.26±4.48*	89.71±2.61*

Values are expressed as mean ± SEM (Number of animals, n=6); significantly different at aP<0.05 when compared with normal control group, *P<0.05 when compared with diabetic control group

Table 2: Determination of biochemical parameters after treatment with LE and ODE of *V. volvacea*

Group	Lipid Profile (mg/dl)			
	Triglyceride	Total Cholesterol	HDL	LDL
Normal control	83.46±3.54	98.42±4.18	63.83±5.41	48.73±5.12
Diabetic control (Streptozotocin)	193.61±5.17a	181.75±4.76 a	25.47±3.47a	173.92±3.45 a
Diabetic + Standard Glibenclamide (0.50 mg/kg)	85.14±4.72*	89.54±3.52*	69.35±6.52*	59.41±4.63*
Diabetic + LE (200 mg/kg)	95.63±6.25*	105.21±3.51*	56.91±2.54*	89.14±6.42*
Diabetic + LE (400 mg/kg)	84.31±2.58*	91.36±4.62*	68.41±6.83*	71.92±4.18*
Diabetic + ODE	115.34±3.14*	135.82±6.38*	35.14±3.17	115.47±5.92*

(200 mg/kg)				
Diabetic + ODE (400 mg/kg)	92.16±6.25	112.67±4.92*	58.37±4.32*	86.29±4.18*

Values are expressed as mean ± SEM (Number of animals, n=6); significantly different at aP<0.05 when compared with normal control group, *P<0.05 when compared with diabetic control group

Table 3: Effect of LE and ODE of *V. volvacea* on changes in body weight in rats

Group	Change in Body weight (gm)		
	Before Induction	After Induction	After Treatment
Normal control	192.15±2.54	194.26±3.47	189.37±2.63
Diabetic control (Streptozotocin)	189.32±3.14	173.82±2.65	145.67±1.72
Diabetic + Standard Glibenclamide (0.50 mg/kg)	181.68±2.69	162.54±3.25	175.92±2.43
Diabetic + LE (200 mg/kg)	193.25±2.52	156.24±5.64	180.41±4.72
Diabetic + LE (400 mg/kg)	203.65±4.62	173.62±2.51	196.17±2.68
Diabetic + ODE (200 mg/kg)	192.47±2.39	162.92±3.19	185.24±4.27
Diabetic + ODE (400 mg/kg)	199.35±1.89	152.69±2.47	188.53±5.92

Values are expressed as mean ± SEM

Table 4: Effect of LE and ODE of *V. volvacea* in insulin level of STZ induced diabetes in rats

Treatment Group	Insulin Level (Mean ±SEM) In mg/dl	
	Initial Reading	Final Reading
Normal control	0.75±0.14	0.79±0.73
Diabetic control (Streptozotocin)	0.83±0.25	0.25±0.54*
Diabetic + Standard Glibenclamide (0.50 mg/kg)	0.81±0.32	0.78±0.18*
Diabetic + LE (200 mg/kg)	0.82±0.25	0.66±0.58*
Diabetic + LE (400 mg/kg)	0.77±0.38	0.77±0.83*
Diabetic + ODE (200 mg/kg)	0.81±0.82	0.70±0.42*
Diabetic + ODE (400 mg/kg)	0.83±0.41	0.76±0.53*

Values are expressed as mean ± SEM (Number of animals, n=6); significantly different at aP<0.05 when compared with normal control group, *P<0.05 when compared with diabetic control group

Table 5: Effect of LE and ODE of *V. volvacea* on liver and renal serum biomarkers of diabetic rats

Treatment Group	AST (U/L)	ALT (U/L)	ALP (U/L)	LDH (U/L)	Urea (mg/dl)	Creatinine (mg/dl)
Normal control	98.26±3.51	53.68±4.27	148.42±3.72	850.31±2.79	49.23±3.45	0.35±0.14
Diabetic control (Streptozotocin)	553.58±2.82a	365.72±3.56 a	614.37±2.41 a	1532.49±4.17 a	123.58±2.48 a	0.82±0.05 a
Diabetic + Standard Glibenclamide (0.50 mg/kg)	82.35±5.14*	78.25±4.15*	126.48±5.37*	828.19±3.65*	43.47±4.31*	0.46±0.08*
Diabetic + LE (200 mg/kg)	125.42±2.71*	138.82±5.17*	185.49±4.82*	932.12±3.28*	77.28±4.54*	0.53±0.58*
Diabetic + LE (400 mg/kg)	95.35±3.35*	61.43±4.25*	124.81±4.17*	852.63±5.39*	52.57±3.28*	0.45±0.41*
Diabetic + ODE (200 mg/kg)	148.62±2.47*	175.28±5.46*	205.41±3.63*	971.43±4.15*	95.72±5.43*	0.59±0.39
Diabetic + ODE (400 mg/kg)	118.75±5.38*	95.14±6.24*	152.93±4.49*	884.54±2.63*	58.44±2.38*	0.50±0.52*

Values are expressed as mean ± SEM (Number of animals, n=6); significantly different at aP<0.05 when compared with normal control group, *P<0.05 when compared with diabetic control group