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Evaluation of anti-diabetic and hypolipidemic effect of ethanolic extract of *Waltheria* indica against streptozotocin induced diabetes in mice

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ABSTRACT

The present study was designed to investigate the anti-diabetic activity of ethanol extract of *Waltheria indica* leaves (EEWI) on streptozotocin induced diabetic mice. Effect of EEWI leaves antidiabetic and hypolipidemic effect was evaluated in streptozotocin (55 mg/ kg, *i.p*) induced hyperglycaemic mice. EEWI (40 and 100 mg/ kg) was administered orally for 15 days. Glibenclamide (1mg/kg, orally for 15 days) was used as reference standard. The diabetic control animals exhibited a significant decrease in body weight compared with control animals. EEWI inhibited streptozotocin-induced weight loss and significantly alter the lipid levels. Administration of the EEWI caused significant dosedependent reduction in serum glucose, cholesterol, triglyceride and glycosylated haemoglobin % levels in streptozotocin induced hyperglycaemic animals. The ethanol extract of *Waltheria indica* leaves exhibited antidiabetic activity might be through increased secretion of insulin and,

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the effect attributed to the presence of flavonoids and phenolic compounds present in extract.

Keywords: Diabetes mellitus, flavonoids, hypolipidemic, hyperglycaemia, streptozotocin

INTRODUCTION

Diabetes mellitus is a social concern. It might even be attributable to inadequate insulin production, or it can be due to insulin's ineffective action, i.e. insulin resistance, or it can be both. Type 1 and type 2 diabetes are the two main forms of diabetes. Insulin Dependent Diabetes Mellitus (IDDM) is another acronym of type 1 diabetes (IDDM). Type 1 diabetes patients have no insulin secretion and must rely on insulin for survival. Type 2 diabetes, also known as Non-Insulin Dependent Diabetes Mellitus (NIDDM), occurs when the pancreas does not produce enough insulin, or when the patient has insulin resistance, or a combination of both. Insulin insufficiency may wreak havoc on a variety of metabolic processes. Diabetes type 2 is more prevalent than diabetes type 1. It is estimated by WHO that an adult suffering from the diabetes mellitus in a low-income family of India, about 25% of the income is being spent.It is studied that 2.8% of world population is affected by diabetes in the year 2000 and it is estimated that it would raise to 366 million in 2030 from 171 million in 2000[1,2]. Sustained diabetes may lead to several consequences like neuropathy, nephropathy, retinopathy and ulcer. The increase in morbid rate due to diabetes is well explained by increase in glycosylated hemoglobin HbA1c[3].

Several approaches have been used to create diabetes mellitus in experimental animals. The pancreatectomy is the most common and successful procedure. Injecting medicines like alloxan or Streptozotocin into animals is the second thing to achieve animal's diabetic[4]. The Langerhans islets beta cells expand and eventually degenerate as a result of these chemicals. Because of its faster inductive rate and lesser toxicity, STZ is recommended over Alloxan for inducing diabetic mice. STZ is a safer alternative to Alloxan. Due to a large reduction in body weight, Alloxan has a greater mortality rate than STZ [5,6]. In rats chemically inoculated with diabetes by Streptozotocin, the last symptoms of insulin insufficiency are easily noticeable [7]. Streptozotocin can trigger an autoimmune process that culminates in the loss of beta cells in the Langerhans islets and can cause beta cell toxicity, resulting in the appearance of clinical diabetes within 2-4 days [8]. We chose the STZ for this research study based on the aforementioned factors.

Waltheria indica (Sterculiaceae.) is a vital medicinal plant distributed throughout tropical forest region in India, and its can be used as sedative, antibacterial, antimalarial, antifungal, anti-anemic, analgesic, antioxidant, anti-convulsant and anti-inflammatory effect [9,10]. The EEWI leaves were found to contain alkaloids, flavonoids, tannins, saponins and cardiac glycosides[11]. These can act as an antioxidant and has potential remedy for diabetes. The project aims at finding out the effect of ethanolic extract of *Waltheria indica* leaves in streptozotocin induced diabetes in Swiss albino mice. As there are no reports specifying this property of this extract in streptozotocin induced diabetic model, hence the project has been carried out.

METHODOLOGY

Plant material and extraction

The leaves of *Waltheria indica* was authenticated and collected from Dr. K.Madhava Chetty, Assistant professor, Department of Botany, Sri Venkateswara University, Tirupati. Cold extraction method using ethanol was used for the extract preparation. The leaves of the plant were sieved and about 100g of the leaves were taken and crushed and later placed in a flask containing 500ml of ethanol.Contents were filtered and filtrate was stored in refrigerator to enhance stability. A second measure of 500 ml of ethanol was run through the residue and left to soak for 24 hrs. Filtrate was collected and stored in the fridge between 0-5°C. The above procedure was repeated for a third time. The filtrates were mixed together and a rotary extractor was used to remove the ethanol from the extract in a 250 ml flask. Flask containing the concentrated extract was stored in a cool dry place until the next stage of experiment.

Animals

Protocols required for performing this study were submitted to the Institutional Animal Ethics Committee (IAEC) during the Animal ethics meeting. After the protocols were approved, healthy animals were procured from the central animal facility for performing the experiments. The CPCSEA approval number for the procured animals is 202/SASTRA/IAEC/RPP. All the animals were maintained in clean polypropylene cages and standard animal diet and water were provided ad libitum. The following table provides the number of mice procured from central animal facility for performing each of the experiments.

Experimental design

A total of 30 Albino mice (25-30g) was used in this study. The mice randomly divided into 5 groups of six animals in each (n=6). Groups are divided as follows:

Group I received with CMC 10ml/kg, *p.o.*; Group II received Streptozotocin at a dose 55 mg / kg, *i.p* ; Group III received Streptozotocin 55 mg/ kg, *i.p* and Glibenclamide 1 mg/kg, *p.o.* ; Group IV & V received Streptozotocin 55 mg/ kg, *i.p* along with EEWI 40 mg/ kg, *p.o.* & EEWI 100 mg/ kg, *p.o.* respectively.

Mice were made diabetic by a single intraperitoneal injection of Streptozotocin, newly dissolved in citrate buffer 0.01 M, pH 4.5 at a dose of 55 mg/kg body weight. The animals were allowed to drink 5% glucose solution overnight after injecting streptozotocin to overcome the drug induced hypoglycemia. Blood glucose levels were measured after 48 hours and animals which had a glucose level greater than 200 mg/dl were considered as diabetic and used for the experiment. After 48hrs of STZ injection the treatment with the plant extract and standard drug was started for 15 days. On 16^{th} day the serum sample were collected for biochemical estimation.

Biochemical estimation

The blood samples were collected from the animals on the 16^{th} day from retro-orbital plexus under mild anaesthesia from overnight fasted animals. Blood samples were centrifuged at 3500 rpm for 15mins to separate the serum from blood. The serum glucose, serum cholesterol and serum triglyceride levels were measured by Bio systems kit and glycosylated hemoglobin % was measured by Monozyme's Glycohemin kit.

Statistical Analysis

The statistical analysis was performed by Graph Pad Prism 5.0 Version (Graph Pad Software, Inc., San Diego, California, USA). All data are presented as Mean ± SEM and comparisons done by one-way ANOVA followed by Tukey's test as a post hoc test values were considered significant at $p<0.05$ or less.

RESULTS and DISCUSSION

Extract yield

The percentage of extract yield was found to be 15.2% when 1500 ml of ethanol was mixed with 100 g of crushed and powdered leaves.

Acute toxicity test

The acute toxicity of plant *of Waltheria indica* was reported that, this plant leaf extract has LD_{50} value of 363 mg/kg of body weight of animal.

Effect of *Waltheria indica* extract on body weight

The EEWI and Glibenclamide did not affected the body weight of the animals,

but significant reduction in body weight was observed in diabetic control animals and are shown in Figure 1. Loss in body weight was observed in streptozotocin induced diabetes mellitus in rats and was controlled by treatment with EEWI. Administration of this extract to hyperglycaemic rats resulted in an increase in body weight compared to streptozotocin induced diabetic rats. The present study findings suggested that EEWI treatment has positive effect on maintaining body weights in diabetic rats. The protective effect of plant extract on body weight of diabetic rats may be due to its ability to reduce hyperglycaemia. A gradual increase in body weights of Glibenclamide treated groups was similar to that of normal control rats. STZ-induced diabetes mellitus was characterized by severe loss of body weight due to increased muscle wasting in diabetes [12].

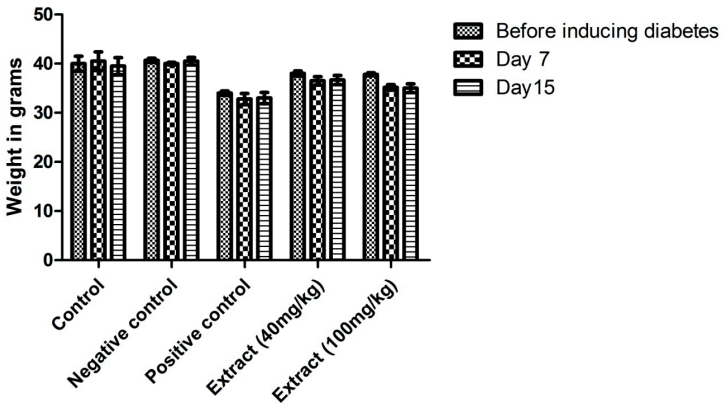

Figure 1. Body weight of the animals before inducing diabetes and on 7th and 15th day of treatment are expressed in grams diabetes using Accu-Check Glucometer.

Effect of *Waltheria indica* extracts on blood glucose levels

The serum glucose level was measured by glucose kit in an auto analyzer[5]. It is evident from the graph that the plant extract is efficient in bringing down the serum glucose level ($p < 0.01$). The blood glucose level of negative control is about 2.5 times greater than that of control Figure 2 & Table 1. The standard drug glibenclamide brought down the serum glucose level by about 58.5 %. The 15th day dose 1 and dose 2 of *Waltheria indica* brought down the serum glucose level by about 49% and 52% respectively and thus can be seen that the extract of *Waltheria indica* provides an effective control in serum glucose level Figure 3.

Table 1. Number of animals procured

S. No	Name of the experiment	No of mice used
1.	Group I: Control group given only CMC (carboxy methyl cellulose) dosage of 0.3ml	6
2.	Group II: Streptozotocin induced diabetic control (55 mg/kg)	6
3.	Group III: Standard drug for diabetes –Glibenclamide (1 mg/kg)	6
4.	Group IV: Effect of Waltheria indica extract on Streptozotocin induced mice at a dose of 40mg/kg	6
5.	Group V: Effect of Waltheria indicaextract on Streptozotocin induced mice at a dose of 100mg/kg	6

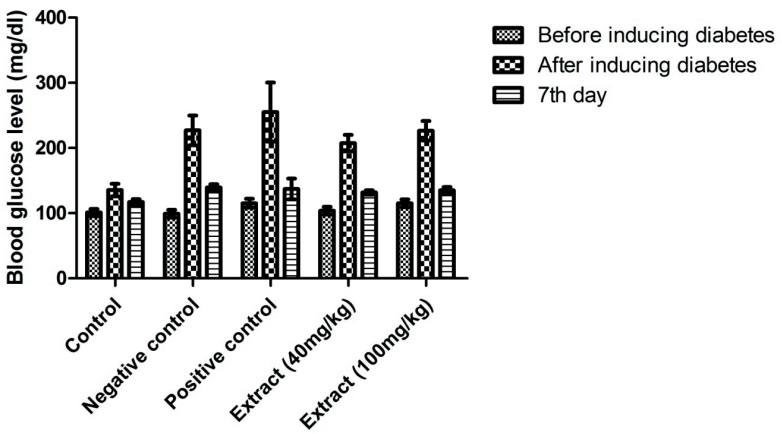

Figure 2. Blood glucose level before and after inducing diabetes and on 7th day of treatment are expressed in mg/dl of *Waltheria indicaextract* on serum glucose levels

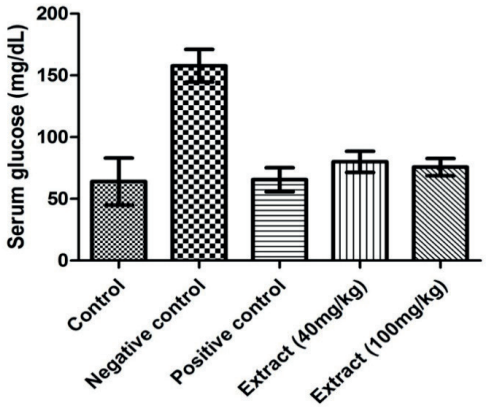

Figure 3. The graph shows comparison between the different groups and their effect on the serum glucose levels of streptozotocin induced diabetic mice.

The antidiabetic effect of *EEWI* may be due to increased release of insulin from the existing β-cells of pancreas. In this context, a number of other plants have also been reported to exert hypoglycaemic activity through insulin release stimulatory effect [13, 14].

Effect of *Waltheria indica* extract on serum cholesterol levels

The serum cholesterol level was measured by cholesterol kit in auto analyzer. It is evident from the graph that the plant extract is not that efficient in bringing down the serum cholesterol level ($P < 0.05$) Figure 4 and Table 1. The induction of diabetes by streptozotocin increased the serum cholesterol level by about 1.5 times. The standard drug brought down the cholesterol level by about 20%. Dose 1 of extract reduced the cholesterol level by 24% and dose 2 of extract reduced cholesterol level by 35%. Thus, the plant extract does not significantly bring down the serum cholesterol but is effective compared with the standard drug glibenclamide. The hypercholesterolemia is associated with Insulin deficiency [15]. The reduction in cholesterol and other lipids in this study was dependent on the concentration of AAWI. It indicates the plant possess the good hypolipidemic effect [16].

Effect of *Waltheria Indica* extract on serum triglyceride levels

The serum triglyceride level was measured by triglyceride kit in auto analyzer. It is evident from the graph that the plant extract is efficient in bringing down the serum cholesterol level ($P < 0.05$) Table 2 & Figure 4. The induction of diabetes by streptozotocin increased the serum triglyceride level by about 2.2 times Figure 5. The standard drug brought down the cholesterol level by about

58%. Dose 1 of extract reduced the cholesterol level by 57.4% and dose 2 of extract reduced cholesterol level by 63%. Thus, the plant extract significantly brings down the serum triglyceride and high dose of extract is more effective than the standard drug glibenclamide.

Table 2. Effect of EEWI on serum glucose, cholesterol, triglycerides and glycosylated in Streptozotocin induced diabetic rat's haemoglobin

Group	Serum glucose(mg/dl)	Serum cholesterol (mg/dl)	Serum triglyceride(mg/dl)	Glycosylated hemoglobin %
Control	64.00 ±19.08	119.00 ±5.29	63.75 ±20.51	6.63 ±0.47
Negative control	157.80 ±13.23	179.00 ±8.00	141.00 ±21.00	10.14 ±0.73
Positive control	65.50 ±9.71	143.30 ±13.49	59.40 ±20.84	6.61 ±0.47
Extract 40mg/kg	80.00 ±8.50	136.00 ±9.38	60.00 ±6.12	7.96 ±0.57
Extract 100mg/kg	75.75 ±7.07	115.50 ±4.66	52.00 ±17.09	6.86 ±0.49

'n = 6' in each group; values are expressed as Mean ± SEM. *p<0.05, ** p<0.01, compared with normal control.. Statistical test employed was one way ANOVA followed by Turkey multiple comparison test.

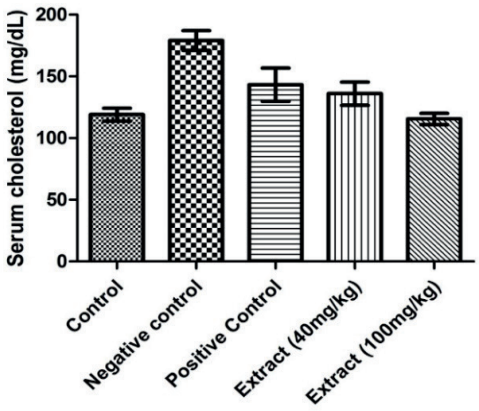

Figure 4. The graph shows comparison between the different groups and their effect on the serum cholesterol levels of streptozotocin induced diabetic mice.

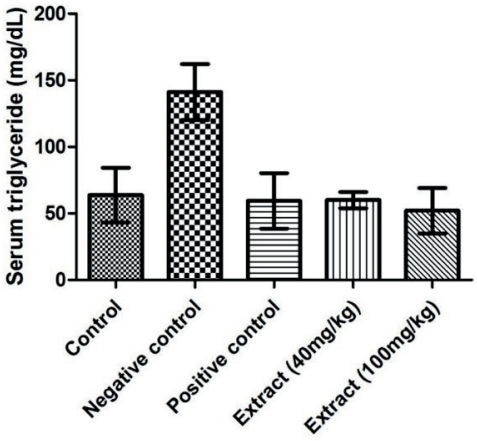

Figure 5. The graph shows comparison between the different groups and their effect on the serum triglyceride levels of streptozotocin induced diabetic mice.

Alterations in lipid concentration, found in 40% of diabetics that similarly were observed in this study in rats with streptozotocin-induced diabetes [17]. Here, the increasing in triglycerides concentrations in diabetic rats it may be due to insulin resistance, an increase in insulin, and glucose intolerance. Treatment with *G. Sylvestre* extract reduced the levels of cholesterol and triglycerides compared with those in their diabetic control groups [18]. The findings of the present work showed that treatment by that *EEWI* caused decreasing in lipid levels like triglycerides in STZ-induced diabetic rats [19].

Effect of *Waltheria indica* extract on glycosylated hemoglobin levels

In diabetic mice, the levels of HbA1C are increased due to the persistent hyperglycemia which results in glycation of hemoglobin. The concentration of HbA1C is related to diabetic retinopathy, nephropathy, and neuropathy and it is considered as a tool for the diagnosis and prognosis of diabetes-associated complications [20]. The synthesis of hemoglobin is reduced in diabetic rats [21]. The present study glycosylated hemoglobin level was measured by glycosylated hemoglobin kit (Monozyme's Glycohemin kit) in auto analyzer. It is evident from the graph that the plant extract is efficient in bringing down the glycosylated hemoglobin% level ($P < 0.05$). The induction of diabetes by streptozotocin increased the glycosylated hemoglobin% level by about 1.5 times Figure 6 & Table1. The standard drug brought down the glycosylated hemoglobin level by about 34%. Dose 1 of extract reduced the glycosylated hemoglobin% level by 32.3% and dose 2 of extract reduced glycosylated hemoglobin level by 21% and restored the glycosylated hemoglobin% to normal level. Thus, the plant extract

significantly bring down the glycosylated hemoglobin and low dose of extract is more effective than the high dose of extract. Plant which has flavonoids, terpenoids, alkaloids, and glycosides have antioxidant activity and claimed to possess antidiabetic effect. Flavonoids present in the plant regenerate the damaged beta cells of pancreases, and the polyphenolic compounds present in the plants produced the hypoglycaemic effects in diabetic rats [22,23]. Antidiabetic effect and hypolipidemic effect of EEWI might be due to presence of flavonoids, polyphenolic compounds in this extract.

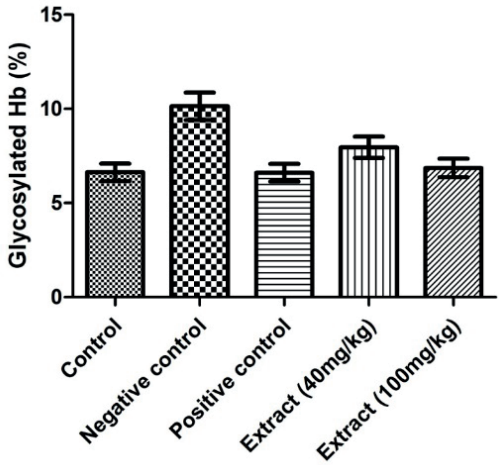

Figure 6. The graph shows comparison between the different groups and their effect on the glycosylated hemoglobin levels of streptozotocin induced diabetic mice.

The present study on the ethanolic extract of *Waltheria indica* leaves possess anti diabetic activity on the streptozotocin induced diabetes in mice by reducing levels of serum glucose, cholesterol, and triglyceride and glycosylated hemoglobin% level. It is also found to be effective in managing the complications associated with diabetes mellitus, such as hypolipidemic, and prevents the defects in lipid metabolism. This might be due free radical scavenging activity through the polyphenols and flavonoids of the plant extract since streptozotocin induces diabetes by increasing hydrogen peroxide. Further studies should be carried out to establish the antidiabetic effect on different animal species, identify the bioactive principle responsible for this effect and also understand the underlying mechanism of cellular actions.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1. Amos A, McCarty D, Zimmet, P. The rising global burden of diabetes and its complications, estimates and projections to the year 2010. Diabet Med**,** 1997; 14: *S1-S85*.

2. King H, Aubert R, Herman W. Global burden of diabetes, 1995 - 2025. Prevalence, numerical estimates and projections. Diabet Car, 1998; 21: 1414 - 1431.

3. Zimmet P. Globalization, coca-colonization and the chronic disease epidemic: can the Doomsday scenario be averted. J Med, 2000; 247: 301 - 310.

4. Ikebukuro K, Adachi Y, Yamada Y, Fujimoto S, Seino Y, Oyaizu H. Treatment of Streptozotocin-induced diabetes mellitus by transplantation of islet cells plus bone Marrow cells via portal vein in rats. Trans, 2002; 73: 512-518.

5. Soltesova D, Herichova I. On the mechanisms of diabetogenic effects of alloxan and streptozotocin. Diabet Meta Endo Vyziva, 2011; 14: 130-138.

6. Lenzen, S. The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabet, 2008; 51: 216-226.

7. Elias D, Prigozin H, Polak N, Rapoport M, Lohse A W, Cohen I R. Autoimmune diabetes induced by the b - Cell toxin STZ. Diabetes, 1994; 43: 992-998.

8. Weiss RB. Streptozocin: A review of its pharmacology, efficacy and toxicity. Can Treat Report, 1982; 66: 427-438.

9. Khaw KT, Wareham N, Luben R, Bingham S, Oakes S, et al. "Glycated haemoglobin, diabetes, and mortality in men in Norfolk cohort of European Prospective Investigation of Cancer and Nutrition (EPIC-Norfolk)." BMJ, 2001, 322, 1-6.

10. Wood DA, Backer G, Faergeman O, Graham J, Mancia G, et al. Prevention of coronary heart disease in clinical practice. Recommendations of the second joint task force of the European Society of Cardiology, European Atherosclerosis Society, and European Society of Hypertension. Eur Hear J, 1998, 19, 1434-503.

11. Olajuyigbe OO, Babalola AE, Afolayan AJ. "Antibacterial and phytochemical screening of crude ethanolic extracts of Waltheria indica Linn. Afr J Microbiol Res, 2011, 5, 3760-3764.

12. Baynes JW, Thorpe SR. Role of oxidative stress in diabetic complications: A new perspective on an old paradigm. Diabetes, 1999; 48:1-9.

13. Jiaa Q, Liub X, Wua X, Wanga R, Hua X, et al. Hypoglycemic activity of a polyphenolic oligomer-rich extract of *Cinnamomum parthenoxyl* bark in normal and streptozotocin induced diabetic rats. Phytomed, 2009; 16: 744-750.

14. Gireesh G, Thomas SK, Joseph B, Paulose CS. Antihyperglycemic and insulin secretory activity of *Costus pictus* leaf extract in streptozotocin induced diabetic rats and in *in vitro* pancreatic islet culture. J Ethno, 2009; 123: 470-474.

15. Tchobroutsky G. Relation of diabetic control to development of microvascular complications. Diabetologia, 1978; 15(3):143.

16. Rodrigues B, Goyal RK, McNeil JH. Effects of hydralazine on STZ-induced diabetic rats-prevention of hyperlipidaemia and improvement in cardiac function. J Pharmacol Exp Therapy, 1986; 237: 299.

17. Ravi K, Rajasekaran S, Subramanian S. Antihyperlipidemic effect of Eugenia jambolana seed kernel on streptozotocin-induced diabetes in rats. Food Chem Toxicol, 2005; 43(9):1433.

18. Zavaroni I, Bonara E, Pagilora M. Risk factors for coronary artery disease in healthy persons with Hyperinsulinemia and normal glucose tolerance. N Engl J Med, 1989; 16: 702-706.

19. Aralelimath V, Bhise S. Anti-diabetic effects of gymnema sylvester extract on streptozotocin induced diabetic rats and possible β-cell protective and regenerative evaluations. Dig J Nanomat Bios, 2012; 7(1):135-142.

20. Palsamy P, Subramanian S. Resveratrol, a natural phytoalexin, normalizes hyperglycemia in streptozotocin-nicotinamide induced experimental diabetic rats. Biomed and Pharmacotherapy, 2008; 62: 598-605.

21. Prabhu KS, Lobo R, Shirwaikar A. Antidiabetic properties of the alcoholic extract of *Sphaeranthus indicus* in streptozotocin-nicotinamide diabetic rats. J of Pharm and Pharmacology, 2008; 60: 909-916.

22. Kannan M, Senthil K, Thiruppathi R, Mandali. Antidiabetic and Antioxidant Properties of Waltheria indica L., an Ethnomedicinal Plant. Inter J of Pharma Research and Health Sciences, 2016; 4: 1376-1384.

23. Lawson EP, Bakoma B, Titrikou AH, Eklu-gadegbéku K, Aklikokou K, et al. Phytochemical screening, antioxidant and hypoglycemic activity of Coccoloba uvifera leaves and Waltheria indica roots extracts. Inter J Pharm Pharma Sci, 2015; 7: 279-283.