

Anti-Diabetic and Anti-Hyperlipidemic Effects of Polyherbal Ethanolic Extracts(*Allium sativum*, *Vinca rosea* & *Mangifera indica*)in Streptozotocin Induced Hyperglycemic Rats

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The investigation of herbal blends may be a new way of producing medication in order to have improved outcomes or advantages in the treatment of diseases.

Abstract:

The investigation of herbal blends may be a different way of producing medication in order to have improved outcomes or advantages in the treatment of diseases. Allium sativum, Vinca rosea and Mangifera indica are three medicinal plants that are already well-known for the treatment of diabetes mellitus, but separately. The combined potent hypoglycemic effect of these plants is expected to achieve the finest antidiabetic effect. This research aims to assess diabetic condition of rats induced by a single dose of 60mg/kg BW streptozotocin intra-peritoneally. The diabetic rats were administered orally with a combination of plant extracts (150mg/kg BW in equal ratio of each extract) for a period of 28 days. Hematological parameters (RBC count, total Hb count), Lipid parameters (TG, serum cholesterol, LDL, VLDL and HDL) and blood glucose levels were evaluated. The study revealed that the lipid parameters and blood glucose levels were profoundly decreased in diabetic rats suggesting the polyherbal extracts as an effective antidiabetic agent.

Keywords:

Polyherbal, Antidiabetic, Streptozotocin, *A.sativum*, *V.rosea*, *M.indica*

Introduction:

Diabetes mellitus can be described as a mixed disorder in the metabolism of carbohydrates and lipids triggering hyperglycemia due to inadequate insulin's action and secretion, or both. (Durgeshnandani *et al*, 2014). Plant based drugs have been known to be safer and cheaper compared to chemically synthesized drugs. Easy obtainability, raw ingesting, also the plant preparations remain

leader of all kinds of sources, with lesser side-effects and cost effectiveness. The plant extracts used in the current study includes the combination of *Vinca rosea*, *Mangifera indica* and *Allium sativum*. The combinational effects of these plants have shown favorable hypoglycemic & hypolipidemic effects against diabetic rats (induced through STZ), considering it in the treatment of diabetes as a potential substitute to oral hypoglycemic agents.

Materials and Methods:

Preparation of plant extractions

Leaves of *Mangifera indica*, *Vinca rosea* and bulbs of *Allium sativum* were collected from the location of Vadavalli area in Coimbatore district. The leaves, bulbs were washed and shade dried for 10-14 days. The leaves and bulbs were pulverized separately to coarse powder (5g) after dried and homogenized with ethanol (50ml each). The powders were solubilized and mixed well (equal ratio) and placed in shaker incubator (29°C) with intermittent stirring for 3 days continually. The prepared plant-solvent mixture was filtered with Whatman filter paper (no.#1). The obtained solvent was placed in petri-plates in hot air oven (50°C-80°C) and allowed for evaporation for 4-5 days. The resulting extracts were collected and preserved in refrigerator for later usage.

Animals:

For the current anti-diabetic study, adult male Albino Wistar rats (42 days aged) ranging between the weight of 150 g – 200 g. Sterile polypropylene cages were used to maintain the rats in a well-ventilated animal house on a continuous routine of light and dark (12 hours each). Ad libitum were made accessible for the animals with standardized rat-pellet diet and clean drinking water. All animal experiments were done in accordance with the approval of the Ethical Committee grant and guidelines for appropriate usage in the laboratory.

Experimental Design: Four group of animals (five in each group) received following treatment schedule via intra-peritoneal route:

Group I (non-diabetic): Normal saline

Group II (diabetic control): STZ (65 mg/kgBW) + nicotinamide 120mg/kg

Group III (standard): STZ(65 mg/kgBW) + nicotinamide 120mg/kg + treated with oral hypoglycemic agent - Glibenclamide 120mg/kg BW

Group IV (treatment): STZ (65 mg/kgBW) + nicotinamide 120mg/kg + treated with Plant extracts (150 mg/kg BW)

Reagents used:

Streptozotocin (S-0130) and Nicotinamide (N-3376) from Sigma-Aldrich, Sodium Citrate (Mw: 294.10), Citric acid (Mw:210.10), Sodium Chloride (0.9%), 10% Sucrose solution were the reagents used for animal experiments.

Induction of Diabetes:

After fasting overnight, initial blood glucose level was tested on the blood withdrawn from the caudal vein. STZ (citrate buffer – pH 4.5) containing Nicotinamide were dissolved in normal saline, was used to induce type 2 diabetes through single dosage of STZ intra-peritoneal injection (60 mg/kg BW) followed by Nicotinamide dosage (120 mg/kg BW). Elevated blood glucose levels were detected after 72 hours, confirming the presence of hyperglycemia. For the experimental analysis, animals possessing greater than 250 mg/dl concentration of blood glucose were used. Blood glucose levels were measured on the day – 0, 7, 14 and 28.

Estimation of Haematological Parameters:

Enumeration of RBC was carried out by the method of Ramnic-sood, 2007 with slight modification. Haemocytometer containing RBC pipette were used to collect mixed blood and transferred onto the counting chamber (0.5 mark & mark II). High power objective (45×) was used to count the RBCs and articulated in terms of cells $\times 10^{12}/l$. Measurement of haemoglobin was done by Sahli's acid haematin method. 0.1N HCL in the Haemoglobinometer (lowest marking) were used to draw up mixed blood of about 20 μ l and made to stand for 10 minutes duration. Colour comparator strip was used to infer the result of reaction mixture after dilution with H₂O.

Estimation of Serum Lipid Parameters:

Total cholesterol level was estimated using Ferric chloride-acetic acid reagent for deproteinization. The contents were centrifuged, supernatant was extracted and added with concentrated sulphuric acid & read colorimetrically (540nm). Triglycerides were estimated using acetyl acetone and meta-periodate reagent. After the addition of Phospho-tungstic acid and Mg ions, the sample was precipitated to obtain Chylomicrons, VLDL and LDL separately. The mixed serum was added with reagent of HDL-Cholesterol precipitating solution readily available in the HDL- kit. The solutions were colorimetrically read at 540nm.

Estimation of Blood Glucose:

“Accu-Chek kit” (Roche diagnostics, USA) glucometer containing GOx or GOD / POx reactive strips were used to estimate the level of glucose withdrawn from the caudal vein of animal.

Statistical analysis:

The findings were shown as mean \pm S.D. ANOVA was carried out to obtain statistical significance (p) and Dunnett multiple comparison test. Value of p (minimum degree) was assumed to be < 0.05; <0.01.

Results:

Table 1: Effects of polyherbal extracts on haematology profile comparison in normal and experimental rats

Treatment	Control	Only STZ	STZ + STD	STZ + Plant extracts
RBC ($\times 10^6/\mu\text{L}$)	5.76 \pm 0.21	5.31 \pm 0.30*	4.78 \pm 0.27*	4.63 \pm 0.46*
Haemoglobin (Hb) (g/dl)	15.47 \pm 0.176	9.71 \pm 0.562*	14.43 \pm 0.273*	14.13 \pm 0.393*

The data are expressed as the mean \pm S.D. Statistical significance (p) calculated by one-way ANOVA followed by Dunnett's, *P < 0.05 calculated by comparing treated group with control group.

The level of RBC is high relative to the diabetic control, while the standard group showed decrease and treatment group showed a significant decrease in the level of RBC compared to diabetic control. Value of Haemoglobin were increased in the diabetic control relative to standard group, whereas, a significant increase was reported in the treatment group relative to diabetic control.

Table 2: Effects of polyherbal; extracts on lipid profile comparison in normal and experimental rats

Treatment	Control	Only STZ	STZ + STD	STZ+Plant extract
	110.5±	103.5±	72.4±	71.47±
Total Cholesterol (mg/dl)	4.91	10.87	8.51*	8.17*
	63±	94.13±	60.73±	81.8±
Triglycerides (mg/dl)	11.98	28.79	9.87	27.43
	70.17±	56.83±	45.1±	39.63±
HDL (mg/dl)	2.858	5.116*	6.275*	4.908*
	43.4±	40±	41.9±	43.2±
LDL (mg/dl)	3.09	1.85*	0.92*	0.35*
	19.73±	23.93±	23.2±	22.9±
VLDL (mg/dl)	0.260	0.441*	1.328*	0.513*

The data are expressed as the mean \pm S.D. Statistical significance (p) calculated by one-way ANOVA followed by Dunnett's, *P< 0.05 calculated by comparing treated group with control group.

The level of cholesterol significantly decreased relative to diabetic control. Value of Triglycerides showed significant decrease in standard group compared to diabetic group however, treatment group showed rise in Triglyceride value compared to non-diabetic control. Value of HDL significantly decreased in treatment group compared to normal rats, while standard group showed decreased value compared to diabetic control. VLDL values were observed to be increased in standard group and decreased in treatment group compared to diabetic control. LDL values were decreased in standard group compared to diabetic control; however, treatment group showed a negligible change compared to diabetic control.

Table 3: Effects of polyherbal extracts on estimation of blood glucose comparison in normal and experimental rats at different time interval

Time interval	Control	Only STZ	STZ + STD	STZ + Plant extract
Day 0	94.2± 2.417	87± 3.899	87± 3.209	93.4± 3.458
Day 3	94.2± 2.417	320± 97.37	313± 121.8	376± 67.42*
Day 10	94.2± 2.417	334± 96.1**	276± 74.14	256.4± 71.34
Day 14	94.2± 2.417	234.2± 95.97*	172±4 9.13	188± 52.48*
Day 28	82.2± 3.98	195.8± 80.13*	58± 23.7	143± 50.67

The data are expressed as the mean ± S.D. Statistical significance (p) calculated by one-way ANOVA followed by Dunnett's, *P< 0.05, **P< 0.01 calculated by comparing treated group with control group.

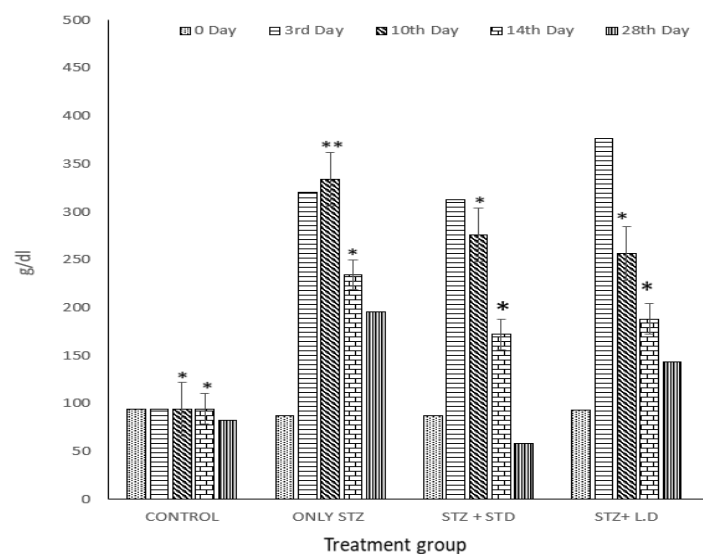


Figure1: Effects of polyherbal extracts on estimation of blood glucose comparison in normal rats and experimental rats at different time interval

The level of blood glucose was reported to increase significantly in treatment group relative to diabetic control on day 3. However, it was observed to increase in standard group while pointedly decreased in treatment group on day 10. There was a constant decrease in both the standard and treatment group compared to diabetic control on day 14. A significant decrease was reported in the treatment group compared to diabetic control on day 28 (end of the experimental period).

Discussion:

The current study has shown that the polyherbal extracts have significant effects on the haematological parameters in streptozotocin induced diabetic condition. It also showed the correlation between hypoglycaemic agents and diabetes mellitus. The plants chosen for the study were listed as anti-diabetic and anti-hyperlipidaemic components in Rizwani *et al*, 2013 and Ganopichayagrai *et al*, 2017. Compared to the standard (Glibenclamide) group, there was a profound drop in the treatment group. Various other studies reported the hypoglycaemic effects of garlic and other plants in line with the present findings (A. Eidi *et al*, 2006). The fall in the level of RBC is also attributed in the study of Chang and Johnson, 1980. The value of Hb count was found to be increased with treatment of plant extracts which is in correspondence to the study of Hela *et al*, 2005. The metabolism involved in hypoglycaemic action directly or indirectly stimulated the secretion of insulin (Gray *et al*, 1999) which increased Hb count for glycosylation.

The administration of polyherbal extracts significantly decreased the value of cholesterol in diabetic animals. Additional studies have confirmed that the treatment with garlic extracts has optimized lipid profile value, such as a fall in serum cholesterol levels, in accordance with the present work (Ahmed *et al*, 2010). The cholesterol reducing tendency was related to the presence of rate controlling enzyme "HMG Coenzyme A reductase" that participated in the cholesterol synthesis metabolism. (Shabani *et al*, 2018). The amount of HDL was also considerably decreased due to the synergistic influence of the hypoglycaemic agent combination. This observation was supported by work of Hanfeld *et al*, 2017. A decrease in the atherogenic index was noticed in treated rats which could be due to the increase in HDL which was similar to the findings of Elango *et al*, 2016. In the current research, rats induced with diabetes through STZ dosages reported a substantial rise in the level of blood glucose, which exhibited the characteristics of Diabetes mellitus. The mixture of plant extracts treated rats showed decreased blood glucose level which is in correspondence with the work of Ghosh *et al*, 2001 for *V. rosea*, Oheari *et al*, 2001 for *A. sativum* and *M. indica*. The results of this study collaborate the advantages of polyherbal therapy articulated by Pournaghi *et al*, 2012. Different mechanism-oriented actions of anti-diabetic potentials of these plants have been proposed by potentiation insulin secretion

in the cells of islets of Langerhans along with its release (Patelet *et al*, 2012) and also due to the presence of various secondary metabolites and active compounds might also have resulted in the hypoglycaemic effect in the combinational extracts (Ebonget *et al*, 2011).

Conclusion:

The ethanolic extracts combination of *Allium sativum*, *Vinca rosea* and *Mangifera indica* showed diminutive impact on serum cholesterol levels, pre-prandial & post prandial values of blood glucose in the Streptozotocin-induced diabetic rats, thus demonstrating an efficient antidiabetic and anti-hyperlipidaemic action. The conclusion of this study confirms the efficacy of various polyherbal combinations that have been experimentally established to support the management of diabetes.

Conflict of interest

All authors declare no conflict of interest.

References:

1. Ahmed MF, Kazim SM, Ghori SS, *et al*. Antidiabetic Activity of *Vinca rosea* Extracts in Alloxan-Induced Diabetic Rats. *International Journal of Endocrinology*. 2010 ;2010:841090. DOI: 10.1155/2010/841090.
2. Antidiabetic effect of garlic (*Allium sativum* L.) in normal and streptozotocin-induced diabetic rats, A. Eidia M, Eidib E, Esmaeilia, *Phytomedicine*; Volume 13, Issues 9–10, 24 November 2006, Pages 624-62.
3. Chang MW, Johnson MA. Effect of garlic on carbohydrate metabolism and lipid synthesis in rats. *J Nutr*. 1980;110:931–936.
4. Effect of *Vinca rosea* extracts in treatment of alloxan diabetes in male albino rats, Ghosh, Sumana, Suryawanshi, S A, Aug-2001, NISCAIR-CSIR, India.
5. Elango, Bakya & Sireesh, Dornadula & Murugesan, Sakthivadivel & Sivasubramanian, Srinivasan & Gunasekaran, Palani & Ramkumar, Kunka. (2016). Anti-hyperlipidemic and anti-peroxidative role of pterostilbene via Nrf2 signaling in experimental diabetes. *European journal of pharmacology*. 777. 10.1016/j.ejphar.2016.02.054.
6. Ganogpichayagrai, A., Palanuvej, C., & Ruangrungsri, N. (2017). Antidiabetic and anticancer activities of *Mangifera indica* cv. Okrong leaves. *Journal of advanced pharmaceutical technology & research*, 8(1), 19–24.
7. Gray AM, Flatt PR. Insulin - releasing and insulin -like activity of the traditional anti-diabetic plant *Coriandrum sativum* (coriander) *Br J Nutr*. 1999;81:203–208.

8. Hanefeld M, Traylor L, Gao L, Landgraf W. The use of lipid-lowering therapy and effects of antihyperglycaemic therapy on lipids in subjects with type 2 diabetes with or without cardiovascular disease: a pooled analysis of data from eleven randomized trials with insulin glargine 100 U/mL. *Cardiovasc Diabetol*. 2017 May 19;16(1):66. doi: 10.1186/s12933-017-0548-0. PMID: 28526014; PMCID: PMC5438574.
9. Helal, Eman. (2013). *Physiological and Histopathological Studies on Bisphenol-A Compound As Xenoestrogen in Male Albino Rats*. *The Egyptian Journal of Hospital Medicine*. 50. 127 – 136. 10.12816/0000829.
10. Herbal remedies for treatment of hypertension D Nandini, V Sharma, NS Chauhan - *Int J Pharm Sci and Res*, 2010.
11. Modak, M., Dixit, P., Londhe, J., Ghaskadbi, S., & Devasagayam, T. P. (2007). Indian herbs and herbal drugs used for the treatment of diabetes. *Journal of clinical biochemistry and nutrition*, 40(3), 163–173.
12. Ohaeri, O.C. Effect of Garlic Oil on the Levels of Various Enzymes in the Serum and Tissue of Streptozotocin Diabetic Rats. *Biosci Rep* 21, 19–24 (2001).
13. P.E. Ebong, I.J. Atangwho, E.U. Eyong, G.E. Egbung, E.V. Ikpeme. Effect of co-administration of extracts of *Vernonia amygdalina* and *Azadirachta indica* on lipid profile and oxidative stress in hepatocytes of normal and diabetic rats. *Agriculture and Biology Journal of North America [Full Article – PDF]* pp. 1087–1095.
14. Patel, D. K., Prasad, S. K., Kumar, R., & Hemalatha, S. (2012). An overview on antidiabetic medicinal plants having insulin mimetic property. *Asian Pacific journal of tropical biomedicine*, 2(4), 320–330.
15. Pournaghi, P., Sadrkhanlou, R. A., Hasanzadeh, S., & Foroughi, A. (2012). An investigation on body weights, blood glucose levels and pituitary-gonadal axis hormones in diabetic and metformin-treated diabetic female rats. *Veterinary research forum: an international quarterly journal*, 3(2), 79–84.
16. Shabani E, Sayemiri K, Mohammadpour M. The effect of garlic on lipid profile and glucose parameters in diabetic patients: A systematic review and meta-analysis. *Prim Care Diabetes*. 2019 Feb;13(1):28-42. doi: 10.1016/j.pcd.2018.07.007. Epub 2018 Jul 23. PMID: 30049636.
17. Syed Ibrahim Rizvi; Neetu Mishra *Traditional Medicine in Management of Type 2 Diabetes Mellitus; Hindawi Journals; Volume 2013; Article ID 712092 ;*