Biochemical
Communication

BBRC
Bioscience Biotechnology
Research Communications

Biosc.Biotech.Res.Comm. Vol 13 No (4) Oct-Nov-Dec 2020 Pp 1879-1885

Methanolic Extract of *Phyllanthus niruri* Ameliorates Certain Biochemical and Metabolic Changes in Streptozotocin-Induced Diabetic Mice

Vinay Bhushan Kumar*[1], Shashi Bhushan Lal[2], Kumud Ranjan Thakur[3] and Ashish Kumar Ghosh[4]

*[1]*TPS College, Patliputra University, Patna, India*
[2]*Department of Zoology, Patna University, Patna, India*
[3,4]*Department of Biochemistry, Patna University, Patna, India*

ABSTRACT

Diabetes mellitus metabolic disorder with impaired carbohydrate, fat, and protein metabolism characterized by increased blood glucose levels. Diabetes being the multifactorial disorder is represented with various organ and metabolic irregularities. Of all those, irregularities of Liver, Kidney, Pancreas, and lipid metabolism are common. Rosiglitazone, thiazolidinediones which are a synthetic drug having various side effects was used for comparative analysis with the desired botanical extract which is safe, having lesser or no side effects and less expensive. Based on folkloric usage and reported literature the present study aimed to investigate the whole plant extract of *Phyllanthus niruri* Linn. (Euphorbiaceae) for biochemical, enzymatic, and enhanced glucose utilization properties on *Swiss albino* mice. The mice were divided into five groups namely, STZ Induced diabetic control mice (35mg/ kg. body weight) non-diabetic control mice, Diabetic treated (DT150) (150 mg/ kg of body wt. extract), Diabetic treated (DT250) (250 mg/ kg of body wt. extract), Diabetic treated (DTRGZ) (2mg/ kg of body wt. rosiglitazone). The Tukey–Karmer Post Hoc test was applied to identify significance among groups. Graphs are plotted using MATLAB version 7.8.0. The extract of *Phyllanthus niruri* showed better outcomes and the result obtained was statistically significant. The result showed better restoration of those biochemical parameters like Glucose, ALT, AST, urea, creatinine, and lipid profiles. It was found that the extract of 250mg/kg. body weight was more effective than other groups and hence the research recommends the exogenous use of the *P. niruri* could be the best candidate to regulate the diabetic complications however, translational research with a large sample size is required.

KEY WORDS: DIABETES MELLITUS, STREPTOZOTOCIN, PHYLLANTHUS NIRURI, SWISS ALBINO, ROSIGLITAZONE.

INTRODUCTION

The human population has always been plagued by diseases that have adversely affected their health and well-being. Of these, one particular disease that causes greater morbidity and mortality, in both young and old people is Diabetes mellitus accompanied with macro vascular and micro vascular complications. It is a metabolic disorder with impaired carbohydrate, fat, and protein metabolism characterized by increased blood glucose levels (Skyler et al., 2017). Statistical analysis of diabetes represents that there are about 463 million

ARTICLE INFORMATION

*Corresponding Author: vinaykumar10121976@gmail.com
Received 30th Sep 2020 Accepted after revision 29th Nov 2020
Print ISSN: 0974-6455 Online ISSN: 2321-4007 CODEN: BBRCBA

Thomson Reuters ISI Web of Science Clarivate Analytics USA and Crossref Indexed Journal

NAAS Journal Score 2020 (4.31) SJIF: 2020 (7.728)
A Society of Science and Nature Publication,

Online Contents Available at: http//www.bbrc.in/
DOI: http://dx.doi.org/10.21786/bbrc/13.4/37

adults having age between 20-79 years who are suffering from this disease which is projected to increase by 700 million by 2050. Its propensity is increasing in low and middle-income countries which are 3 in 4 (79%) are diabetes sufferers. It is estimated that 1 in 5 people (136 million) of age above 65 years are having diabetes and 232 million are undiagnosed (IDF, 2019). Diabetes caused 760 billion USD expenditure in 2019 which is 10% of the total health expenditure. More than 20 million live births (1 in 6 births) are affected by hyperglycemia and out of this 84% developed gestational diabetes mellitus. 764 million candidates are at increased risk of developing diabetes globally (IDF, 2019).

Diabetes being the multifactorial disorder is represented with various organ and metabolic irregularities. Of all those, irregularities of the Liver, Kidney, Pancreas, and lipid metabolism are common. It is associated with hepato-renal and lipid abnormalities which are reflected by altered organs and its metabolic activities (Sirovina et al., 2016). Metabolic diseases like Non-alcoholic fatty liver disease (NAFLD) are the most common which accounts for 70-80% diabetes sufferer. The co-existence of Diabetes and NAFLD sometimes causes serious consequences leading to a severe form of NAFLD and chronic vascular complications of Diabetes mellitus (Targher et al., 2018; Elhence et al., 2020).

Recent research conducted on human and animal models strongly supports the concept that the potent reason for diabetic nephropathy is associated with altered metabolism of glucose and persistent hyperglycemia. Oxidative stress is known to play a significant role in the induction of these abnormalities (Miranda-Diaz et al., 2016). High levels of oxidative stress with the excessive generation of free radicals and depleted levels of free radical–scavenging enzymes have been demonstrated in several studies, both in experimental animal models and in humans with diabetes (Rahman et al., 2017; Yaribeygi et al., 2020).

It had been shown that the presence of diabetes encourages enhanced cardiovascular diseases (CVD) risk as compared to non-diabetes with marked complications like heart failure, peripheral arterial disease, and coronary heart disease (Glovaci et al., 2019). Patients with type 2 diabetes (T2D) show the widespread prevalence of lipid abnormalities, contributing to cardiovascular diseases. Diabetic dyslipidemia includes quantitative, qualitative, and kinetic lipoprotein abnormalities, which, altogether, cause an imbalanced atherogenic lipid profile (Gupta et al., 2019).

The market is flooded with different kinds of mono target drugs with good potential. In spite of excellent vigor, these synthetic anti-diabetic drugs had presented undesirable therapeutic reputation marked with fluid retention, hypoglycemia, liver hepatic issues, lactic acidosis, weight gain, and cardiac hypertrophy. Rosiglitazone, a thiazolidinedione in the present study was used for comparative analysis with the desired botanical extract. It behaves widely as an insulin sensitizer by altering the transcriptional activity of PPARγ. Meanwhile, Food and Drug Administration (FDA) had severely restricted its use because of weight gain, fluid retention, bone fractures, and the associated increase in congestive cardiovascular complications (Kahn et al., 2010; Lebovitz et al., 2019).

Thus, a collaborative translational effort for the search of more effective medicine for T2DM has become the need of the time regarding the safety and efficacy due to the unavoidable side effects of synthetic drugs. For example, metformin, less toxic biguanides, and the potent oral hypoglycemic agent was developed from the *Galega officinalis* plant and used to regulate hyperglycemia. Based on folkloric usage and reported literature the present study aims to investigate the whole plant extract of *Phyllanthus niruri* Linn. (Euphorbiaceae) for Biochemical, enzymatic, and enhanced glucose utilization properties. Earlier it was showed that the extract of this plant has strong anti-oxidative and anti-diabetic properties due to the presence of active phytochemical components like polyphenols, tannin, and flavonoid. *In-vitro* and *in-vivo* studies have provided scientific evidence, whereby administration of *P. niruri* leaf aqueous extract to diabetic rats reduced oxidative burden in the kidney by preventing the depletion of endogenous antioxidant enzymes (Swapna et al., 2014). In a recent study, the cardioprotective action of aqueous extracts of *P. amarus* was studied against high-sugar (fructose) diet-mediated cardiac damage in Wistar rats (Bailey et al., 2017).

Following 60-days of sugar diet, and Co-administration of *P. amarus* aqueous extracts for 60 days impeded cardiac and aortic lipids profile and decreased phospholipid formation (Putakala et al., 2017). The aqueous extracts of *P. niruri* were extensively studied and it was found that due to the presence of phenolic components in the extract displayed the anti-diabetic properties (Singh et al., 2017). In another study with the extract of *P. niruri* demonstrated that it lowers the Diabetic complications like obesity, oxidative stress by decreasing glucose concentration which decreases glycosylation and hence decreased oxidative stress and lipid levels in the blood of obese diabetic rats (Mediani et al, 2016). Recent research evidences *In vivo* administration of aqueous extract of *P. niruri* in rats (25, 50, 100 and 200 mg/kg) caused normalization of AST, ALT, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), total cholesterol (TC), triglycyrides (TG), total bilirubin (TB), glucose, total proteins (TP), urea and creatinine levels which were elevated by carbon tetrachloride (Ezzat et al., 2020). Based on the previous studies a comprehensive study was designed to trace the efficacy of the synthetic drugs and botanicals synergistically.

MATERIAL AND METHODS

This study includes whole plant extract of *Phyllanthus niruri* Linn. (Euphorbiaceae) which was collected from Science College Campus of Patna University, Patna, Bihar, India. It was further identified and botanically authenticated according to the relevant monographs

of Indian Pharmacopoeia (2012) and the same has been deposited in the Bio-chemistry Department, Patna University, Patna, Bihar, India. For animal studies, the research study was carried out on the albino mice weighing around 16–20 gram having 6.4 ± 0.5 cm lengths. The mice were housed in polypropylene cages and fed on normal lab made chow and maintained under standard environmental conditions (21±2°C, 55±5% humidity, 12 hr Light: Dark cycle).

To study the induction of diabetes, the Streptozotocin (STZ) is purchase from Merck was dissolved in 1mMolar citrate buffer at the pH 4.5 and always prepared fresh for immediate use (within 5 minutes). Diabetes was induced by multiple intra-peritoneal injection of freshly prepared STZ solution in 0.05 M sodium citrate (pH 4.5) at the dose of 35 mg/kg body weight followed by fasting (Andrade et al., 2016).

To study the animal groupings and experimental design, the mice were divided into five groups having six mice in each group:

Group – I STZ Induced diabetic control mice receiving citrate buffer only
Group II non-diabetic control mice receiving only citrate buffer solution
Group III Diabetic treated (DT150) receiving of 150 mg/ kg of body wt. extract
Group IV Diabetic treated (DT250) Receiving 250 mg/ kg of body wt. extract
Group V Diabetic treated (DTRGZ) Receiving 2mg/ kg of body wt. Rosiglitazone

For the extract preparation, freshly harvested plant samples were washed under running tap water, blotted with filter paper and was dried in the shade at room temperature. The dried plant sample (2.6 kg) was then soaked with absolute methanol under reflux condition for the methanolic extract preparation. The sample was then homogenized with extraction buffer and the supernatant collected after three rounds of extraction. The solvent was evaporated under reduced pressure in a rotary evaporator at 40 ^{0}C. To this thick paste colloidal silicon dioxide was added and dried in vacuum tube dryer. The obtained plant extract was stored in freezer at -20^0 C until further test.

For the Biochemical estimation, the desired Biochemical parameters were accessed to monitor the metabolic activity of the mice in the respective groups.Fasting Plasma Glucose by GOD POD method (Trinder, 1969), serum Cholesterol CHOD POD , triglyceride uding GPO method, HDL by Phosphotungestic method (Burstein et al.,1970). Serum LDL and VLDL were calculated using Frieldwald formula, serum creatinine by alkaline picrate method (Jaffe, 1986), Serum urea by Nitroprussic method, Alanine aminotransferase (ALT) Reitman and Frankel method and Aspartate aminitransferases (AST) Modified IFCC method (Schumann et al., 2002).

For the statistical analysis, data were expressed as the mean ± S.E.M. For statistical analysis of the data, group means were compared by one-way ANOVA (analysis of variance) with *Post Hoc* analysis. The Tukey–Karmer Post Hoc test was applied to identify significance among groups. Graphs are plotted using MATLAB version 7.8.0 R2009a, Natick, Massachusetts: The Mathworks Inc. 2009.

RESULTS AND DISCUSSION

Table 1. Body weight changes in mice

Groups	Day 0	Day 7	Day 15
Normal control (NC)	18.95 ± 2.76	20.80 ± 2.39	23.89 ± 2.20
Diabetic control (DC)	10.70±1.05	09.45±0.95	8.47±1.32
P. niruri extract (150 mg/kg) (DT150)	10.71±2.03*	12.11±1.67*	13.72±1.53*
P. niruri extract (250 mg/kg) (DT250)	10.68±1.63*	12.74±2.37*	14.84±2.67*
Rosiglitazone (2 mg/kg) (DTRGZ)	10.70 ± 3.74*	14.82 ± 3.91*	16.04 ± 1.84*

Values expressed as Mean ± S.E.M, n = 6 in each group; *Significant as compared to control.

Effect of *P. niruri* extract on body weight: The diabetic control (DC) mice presented significantly lower body weight ($p<0.001$) when compared with the normal control (NC) mice which indicates towards the stress condition of the mice (Xu et al., 2017). A significant body weight gains were observed in the treated groups of diabetic mice (DT150 and DT250) as compared to the DC ones. The DT150 and DT250 group showed an increase of 28% and 39% in body weight respectively after 15 days of treatment. Contrary to this, DTRGZ group mice showed

an increase of 50% in body weight after 15 days of treatment (Table 1). Extract of the *P. niruri* reduced the diabetic complication and reordered the metabolic activity in mice resulted into restoration of the body weight (Wat et al., 2018).

Effect of *P. niruri* extract on blood glucose level: The changes in the blood glucose levels before and after receiving the treatment in normal and diabetic mice are listed in Table. As expected, the DC mice showed significantly ($p<0.001$) higher level of glucose (+278%), when compared with their normal control counterparts. Diabetic mice of both of the groups (DT150 and DT250) showed a reduction in glucose levels when compared to the DC ones; nevertheless, the reduction was particularly evident in the DT250 mice (–50%; $p<0.001$).

Table 2. Effects of different doses of *P. niruri* extract and rosiglitazone on blood glucose levels in mice.

Groups	Blood glucose levels (mmol/l) in week				
	Pretreatment	Post-treatment			
	0	1	2	3	4
Normal control (NC)	3.95 ±0.13**	4.07 ± 0.14**	4.06 ± 0.25**	4.05 ± 0.16**	3.99 ± 0.19**
Diabetic control (DC)	14.96 ± 1.55*	14.91 ± 1.48*	14.78 ± 1.59*	14.96 ± 1.49*	14.94 ± 1.48*
P. niruri extract (150 mg/kg) (DT150)	14.97 ± 1.40	13.04 ±1.18*	10.64 ±2.09**	9.69 ± 1.28**	9.27 ± 1.79**
P. niruri extract (250 mg/kg) (DT250)	14.64 ± 1.59	11.86 ± 1.38**	9.65 ± 1.28**	8.27 ± 1.74**	8.15 ±1.28**
Rosiglitazone (2 mg/kg) (DTRGZ)	15.03 ± 1.49	9.84 ± 1.48**	5.57 ± 1.28**	4.97 ± 1.35**	4.94 ± 0.97**

* $p<0.05$ as compared with normal control. ** $p < 0.01$ as compared with diabetic control.

Table 3. Effects of different doses of *P. niruri* extract and rosiglitazone on serum lipid levels in mice.

Groups	TC (mmol/lit)	TG (mmol/lit)	HDL (mmol/lit)	HDL/TC (%)	LDL (mmol/lit)
Normal control (NC)	4.15 ±0.86**	1.14 ±0.09**	2.86 ±0.29**	68.91 ±4.66**	0.27 ±0.04**
Diabetic control (DC)	9.84 ±1.56*	1.96 ±0.29*	1.31 ±0.58*	13.31 ±1.97*	0.96 ±0.16*
P. niruri extract (DT150) (150 mg/kg)	7.23 ±0.44**	0.87 ±0.08**	2.54 ±0.36	35.13 ±3.37**	0.47 ±0.06**
P. niruri extract (250 mg/kg) (DT250)	6.42 ±0.64**	0.98 ±0.17**	2.78 ±0.46**	43.39 ±4.26**	0.44 ±0.07**
Rosiglitazone (2 mg/kg) (DTRGZ)	6.58 ±1.35**	1.77 ±0.17**	2.90 ±0.55**	44.07 ±5.56**	0.41 ±0.09**

* $p<0.05$ as compared with normal control. ** $p < 0.01$ as compared with diabetic control.TC-total cholesterol, TG-total glycerol, HDL- high density lipoprotein, LDL-Low density lipoprotein.

When compared, the glucose levels of the DT250 versus the DC group mice during the 4-week treatment program, a significantly lower value in the first was also found (–68%; $p<0.001$). Nevertheless, this drop in the glucose levels was more evident in the DT150 rats (–57%) than in the DT250 mice. In contrast to this, DTRGZ group mice showed almost 100% drop in glucose level after 4-weeks of the treatment program (Table 2). These findings of the botanical extract of *P. niruri* may be indebted to their blood glucose-lowering properties to inhibition of glucose absorption and enhancement of glucose storage and utilization. The findings are in line with the previous study (Okoli et al., 2011; Thakur et al., 2016).

Effect of *P. niruri* extract on lipid profile: When compared with normal control, the diabetic mice had higher total cholesterol (TC) (+137%; $p<0.001$) and TGs (+72%; $p<0.001$) values (Table 3). These changes in biochemical parameters are as expected, as when the uncontrolled diabetic status progresses, substantial changes in total

cholesterol and triglycerides values are predictable. Diabetic mice treated with lower dose of *P. niruri* extract (DT150) showed significantly lower values of serum TC (-26.5%; p<0.001) and TGs (-55.6%; p<0.001), when compared with the DC counterparts. The DT250 treatment showed superior lowering effects compared with the DC counterparts as well as DT150 group mice by (-34.7%; p<0.001) on serum TC levels and (-50%; p<0.001) on TGs levels (Table). Contrarily, treatment with rosiglitazone (DTRGZ) showed (-33.2%; p<0.001) on TC levels and (-09.6%; p<0.001) on TGs levels compared with diabetic control mice (Table 3).

Relative to normal control, the diabetic mice had higher value of low-density lipoprotein (LDL) (+250%; p<0.001) while diminished value of high-density lipoprotein (HDL) (-54%; p<0.001) (Table). This is because when the unrestrained diabetic condition advances, considerable changes in these biochemical parameters are as expected and predictable. Diabetic mice treated with lower dose of *P. niruri* extract (DT150) showed significantly lower values of serum LDL (-62.1%; p<0.001) and higher value of HDL (-48.4%; p<0.001), when compared with the DC counterparts (Table).

All over again, the DT250 treatment showed even better lowering effects on LDL (-34.7%; p<0.001) compared with the DC counterparts as well as DT150 group mice and improved level of HDL (+52.9%; p<0.001) (Table). In contrast, treatment with rosiglitazone (DTRGZ) showed a considerable diminished level of LDL (-175%; p<0.001) while improved level of HDL (+70.5%; p<0.001) compared with diabetic control mice (Table 3). The explanation for the present investigation is that *P. niruri* may prevent hyperlipidemia by decreasing lipid accumulation and the finding is in consistent with (Mediani et al., 2016).

Table 4. Blood urea, creatinine, ALT & AST in all groups before and after treatment with *P. niruri* extract.

Groups	Urea (mg/dl)	Creatinine (mg/dl)	ALT (IU/L)	AST (IU/L)
Normal control (NC)	38.15 ± 0.44**	0.89 ± 0.038**	28.47 ± 0.48**	67.13 ± 1.43**
Diabetic control (DC)	90.45 ± 1.73*	1.48 ± 0.037*	63.75 ± 1.74*	112.42 ± 2.43*
P. niruri extract (150 mg/kg) (DT150)	48.22 ± 1.15**	1.24 ± 0.054**	32.85 ± 2.94**	63.48 ± 0.75**
P. niruri extract (250 mg/kg) (DT250)	37.79 ± 2.36**	1.06 ± 0.026**	27.74 ± 0.55*	63.87 ± 1.45*
Rosiglitazone (2 mg/kg) (DTRGZ)	38.57 ± 0.15	0.97 ± 0.025	30.94 ± 0.57	73.47 ± 1.76

* p<0.05 as compared with normal control. ** p < 0.01 as compared with diabetic control.

Effect of *P. niruri* extract on kidney function and liver function markers: Diabetic mice have higher levels (approximately twice) of blood urea, creatinine, SGOT, SGPT. All four markers decrease considerably in Rosiglitazone treated diabetic mice (DTRGZ) when compared to diabetic control mice (DC). In DTRGZ mice the parameters, serum urea, serum creatinine, serum SGPT, and serum SGOT were reduced by 134%, 52%, 106%, and 53% respectively (Table 4) Treatment with *P. niruri* extract decreases the values of all the four markers in a dose-dependent manner when compared to diabetic control mice.

The maximum efficacious dose was found to be 250 mg/kg body weight of mice (Table 4). Thus, the result showed that the *P. niruri* extract is also as effective as rosiglitazone in improving kidney function. The extract of *Phyllanthus niruri* helps to preserve kidney function towards normal by ameliorating histopathological changes through reduction of, inflammation, fibrosis, and apoptosis in diabetic mice. The present results corroborate with the previous findings (Giribabu et al., 2017).

CONCLUSION

The plant under investigation, *P. niruri* whole plant extract is anti-diabetic due to the presence of different types of active components, which may have different mechanisms of action which reflects with the restored concentration of glucose, organ function enzymes, and lipid level. Treatment of diabetic albino mice with the two different doses of *P. niruri* extracts showed a dose-dependent differential protective effect on liver function tests and kidney function tests. Extract of *P. niruri* also reduced the secondary complications differentially, including cardiovascular diseases, insulin resistance, and atherosclerosis caused due to hyperlipidemia in diabetic rats. Therefore, relying on botanical phytochemicals as an anti-diabetic therapy may be beneficial and this could be considered as a safe supplementary therapy for long-term and effective management of diabetic patients.

ACKNOWLEDGEMENTS

Authors are thankful to the Department of Biochemistry, Patna University for providing necessary equipment

and Mr. Ravinder for helping in research activities in the Department.

Conflict of Interest: Authors declares no conflict of interest regarding publication or any other activity related to this article.

REFERENCES

Andrade EF, Lima AR, and Nunes IE, (2016). Exercise and Beta-Glucan Consumption (*Saccharomyces cerevisiae*) Improve the Metabolic Profile and Reduce the Atherogenic Index in Type 2 Diabetic Rats (HFD/STZ). Nutrients. 8(12):792.

Bailey CJ (2017). Metformin: historical overview. Diabetologia. 60(9):1566-1576.

Burstein M, Scholnick HR, and Morfin R (1970). Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. J Lipid Res. 11(6):583-595.

Elhence A, and Shalimar (2020). Treatment of non-alcoholic fatty liver disease - Current perspectives. Indian J Gastroenterol. 39(1):22-31.

Ezzat MI, Okba MM, Ahmed SH, El-Banna HA, Prince A, Mohamed SO, and Ezzat SM (2020). In-depth hepatoprotective mechanistic study of *Phyllanthus niruri*: In vitro and in vivo studies and its chemical characterization. PLoS One. 15; 15(1): e0226185.

Giribabu N, Karim K, Kilari EK, and Salleh N (2017). *Phyllanthus niruri* leaves aqueous extract improves kidney functions, ameliorates kidney oxidative stress, inflammation, fibrosis and apoptosis and enhances kidney cell proliferation in adult male rats with diabetes mellitus. J Ethnopharmacol. 205: 123-137.

Glovaci D, Fan W, and Wong ND (2019). Epidemiology of Diabetes Mellitus and Cardiovascular Disease. Curr Cardiol Rep. 21(4):21

Gupta M, Tummala R, and Ghosh RK, (2019). An update on pharmacotherapies in diabetic dyslipidemia. Prog Cardiovasc Dis. 62(4):334-341.

International Diabetes Federation (2019). IDF Diabetes Atlas, 9th edn. Brussels,Belgium. Available at: https://www.diabetesatlas.org

Jaffe, M. (1886). A new reaction of creatinine in normal generated urine with picric acid and its precipitation products. Zeitschrift fur Physiologische Chemie, 10, 391– 400.

Kahn BB, and McGraw TE (2010). Rosiglitazone, PPARγ, and type 2 diabetes. N Engl J Med. 363(27):2667-2669.

Lebovitz HE (2019). Thiazolidinediones: The Forgotten Diabetes Medications. Curr Diab Rep. 19(12):151.

Mediani A, Abas F, and Maulidiani M, (2016). Metabolic and biochemical changes in streptozotocin induced obese-diabetic rats treated with *Phyllanthus niruri* extract. J Pharm Biomed Anal. 128:302-312.

Mediani A, Abas F, Maulidiani M, Khatib A, Tan CP, Ismail IS, Shaari K, Ismail A, and Lajis NH (2016). Metabolic and biochemical changes in streptozotocin induced obese-diabetic rats treated with *Phyllanthus niruri* extract. J Pharm Biomed Anal. 128():302-312.

Miranda-Díaz AG, Pazarín-Villaseñor L, Yanowsky-Escatell FG, and Andrade-Sierra J (2016). Oxidative Stress in Diabetic Nephropathy with Early Chronic Kidney Disease. J Diabetes Res. 2016:7047238.

Okoli CO, Obidike IC, Ezike AC, Akah PA, and Salawu OA (2011). Studies on the possible mechanisms of antidiabetic activity of extract of aerial parts of *Phyllanthus niruri*. Pharm Biol. 49(3):248-255.

Putakala M, Gujjala S, Nukala S, Bongu SBR, Chintakunta N, and Desireddy S (2017). Cardioprotective effect of *Phyllanthus amarus* against high fructose diet induced myocardial and aortic stress in rat model. Biomed Pharmacother. 95():1359-1368.

Rehman K, and Akash MSH (2017). Mechanism of Generation of Oxidative Stress and Pathophysiology of Type 2 Diabetes Mellitus: How Are They Interlinked? J Cell Biochem. 118(11):3577-3585.

Schumann G, Bonora R, Ceriotti F, Ferard G, Ferrero CA, and Franck PF, (2002). IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37 degrees C. International Federation of Clinical Chemistry and Laboratory Medicine. Part 5. Reference procedure for the measurement of catalytic concentration of aspartate aminotransferase. Clin Chem Lab Med. 40:725–33.

Singh S., Chauhan M.G., Kaur B., Kumar B., Gulati M., and Singh S.K (2017). Characterization, organoleptic evaluation and standardization of aqueous extracts of antidiabetic herbs *Trigonella foenum*, *Allium sativum, Aloe vera, Phyllanthus niruri*. J. Pharm. Res. 11:1370–1375.

Sirovina D, Oršolic N, Gregorovic G, and Koncic MZ (2016). Naringenin ameliorates pathological changes in liver and kidney of diabetic mice: a preliminary study. Arh Hig Rada Toksikol. 67(1):19-24.

Skyler JS, Bakris GL, and Bonifacio E, (2017). Differentiation of Diabetes by Pathophysiology, Natural History, and Prognosis. Diabetes. 66(2):241-255.

Swapna RS, and Salleh N (2014). Aqueous Extract of *Phyllanthus niruri* Leaves Displays In Vitro Antioxidant Activity and Prevents the Elevation of Oxidative Stress in the Kidney of Streptozotocin-Induced Diabetic Male Rats. Evid Based Complement Alternat.: 834815.

Targher G, Lonardo A, and Byrne CD (2018). Nonalcoholic fatty liver disease and chronic vascular complications of diabetes mellitus. Nat Rev Endocrinol.

14(2):99-114.

Thakur KR, Padmadeo SR, Mishra BB, and Pranay K (2016). Study of ameliorating properties of *Tinospora cordifolia* on Diabetes and acute Pancreatitis in Alloxan treated rats. 8(18):133-140.

Trinder P (1969). Determination of Glucose in Blood Using Glucose Oxidase with an Alternative Oxygen Acceptor. Annals of clinical Biochemistry. 6; 24.

Wat E, Wang Y, and Chan K, (2018). An *in vitro* and *in vivo* study of a 4-herb formula on the management of diet-induced metabolic syndrome. Phytomedicine. 42:112-125.

Xu, F., Wang, N., Li, G., Guo, W., Yang, C. and Liu, D., (2017). Establishment and Assessment of Mice Models of Type 2 Diabetes Mellitus. Zhongguo yi xue ke xue yuan xue bao. Acta Academiae Medicinae Sinicae, 39(3), p.324.

Yaribeygi H, Sathyapalan T, Atkin SL, and Sahebkar A (2020). Molecular Mechanisms Linking Oxidative Stress and Diabetes Mellitus. Oxid Med Cell Longev. 2:8609213. doi: 10.1155/2020/8609213.