Preparation And Evaluation of Antidiabetic and Antihyperlipidemic Activities of Polyherbal Formulation in Alloxan Induced Diabetic Rat

Satish kalshetty[1], Dr. Amit kumar[2], Dr. Mohd Rafiq[3]
[1]*Ph.D Research Scholar Mewar University, Rajasthan.*
[2]*Principal and Ph.D. Research Guide, Mewar University Rajasthan.*
[3]*Professor and HOD, MAM College of Pharmacy, Kalaburagi – Karnataka.*

***Abstract*—The present study focuses on the preparation and evaluation of antidiabetic and antihyperlipidemic activities of a polyherbal formulation** in **Alloxan-induced diabetic rats**. **Diabetes mellitus, a chronic metabolic disorder characterized by hyperglycemia and dyslipidemia, poses a significant global health burden. Herbal medicines, owing to their multifaceted therapeutic potential and minimal side effects, have gained prominence in the management of diabetes and associated lipid abnormalities.**

A polyherbal formulation (*Semecarpus anacardium*, *Portulaca quadrifida* and *Acacia nilotica*) was developed using selected medicinal plants traditionally known for their antidiabetic and lipid-lowering properties. The formulation was administered orally to Alloxan-induced diabetic Wistar rats over a specified treatment period. Various parameters including fasting blood glucose levels, body weight, serum lipid profile (total cholesterol, triglycerides, HDL, LDL, VLDL), and glycated hemoglobin (HbA1c) were evaluated to assess the therapeutic efficacy.

The results demonstrated a significant reduction in fasting blood glucose and HbA1c levels, **along with an improvement in lipid profile**, **indicating potent antidiabetic and antihyperlipidemic activities of the polyherbal formulation.**

This study suggests that the formulated polyherbal combination exhibits synergistic action and holds promise as an effective and natural alternative for the management of diabetes and its complications.

***Index Terms*—Polyherbal formulation, Alloxan, Antidiabetic, Hypolipedimic.**

I. INTRODUCTION

Diabetes mellitus is a complex and chronic metabolic disorder characterized by persistent hyperglycemia, resulting from defects in insulin secretion, insulin action, or both. It is one of the most prevalent non-communicable diseases worldwide, affecting millions and leading to serious complications such as cardiovascular diseases, nephropathy, neuropathy, and retinopathy.[1] Among the types of diabetes, Type 1 diabetes is insulin-dependent and caused by autoimmune destruction of pancreatic β-cells, whereas Type 2 diabetes involves insulin resistance and relative insulin deficiency. Additionally, diabetes is frequently associated with dyslipidemia, which significantly contributes to atherosclerosis and other cardiovascular disorders.[2]

Current pharmacological treatments for diabetes include insulin and oral hypoglycemic agents such as sulfonylureas, biguanides, and thiazolidinediones. While these drugs are effective, they are often associated with adverse effects such as hypoglycemia, weight gain, gastrointestinal disturbances, and long-term toxicity.[3] Consequently, there is a growing interest in alternative therapies, particularly those derived from traditional medicinal systems that utilize plant-based formulations.

Herbal medicines have been widely used in traditional systems like Ayurveda, Unani, and Traditional Chinese Medicine for the treatment of diabetes.[4] Many medicinal plants possess bioactive phytoconstituents such as alkaloids, flavonoids, terpenoids, and saponins that have shown antidiabetic and lipid-lowering effects through various mechanisms. Polyherbal formulations, which combine multiple medicinal plants, may exert synergistic effects, enhance therapeutic efficacy, and reduce toxicity.[5]

Alloxan-induced diabetes in experimental animals, especially rats, is a well-established model for studying the pathophysiology of diabetes and evaluating the efficacy of antidiabetic agents.[6] Alloxan selectively destroys insulin-producing β-cells in the pancreas, mimicking Type 1 diabetes and enabling the assessment of both hypoglycemic and β-cell regenerative properties of therapeutic agents.[7]

The present study aims to develop a polyherbal formulation comprising selected medicinal plants traditionally known for their antidiabetic and antihyperlipidemic potential. The formulation will be evaluated in alloxan-induced diabetic rats to investigate its effects on blood glucose levels, lipid profile, body weight, and glycated hemoglobin (HbA1c).

Semecarpus anacardium

Portulaca quadrifida

Acacia nilotica

II. METHODOLOGY

Chemicals:

Alloxan monohydrate, Atorvastatin and Glibenclamide were obtained from Sigma laboratories in Bangalore, while the one touch Glucometer and Blood gluco-strips were procured from Med plus in Kalaburagi.

Additionally, the following reagents were obtained from Sigma in Bangalore: EDTA, NaCl (0.9% w/v), Phosphate buffer with a pH of 6.5 at a concentration of 90 mmol/L, Phenol at a concentration of 26 mmol/L, 4-Aminoantipyrine at a concentration of 0.4 mmol/L, Cholesterol Esterase at a concentration of 500 U/L, Cholesterol Oxidase at a concentration of 500 U/L, Peroxidase at a concentration of 1250 U/L, and Glycerol kinase at a concentration of 1250 U/L. All of the aforementioned reagents are ready for use and exhibit stability up to the expiry date specified on their respective labels when stored at a temperature range of 2-8°C.

Collection of Plant Material:

Fresh whole plant material of (*Semecarpus anacardium*, *Portulaca quadrifida* and *Acacia nilotica*) was collected from the local fields of Kalaburagi. The plant specimen was identified and authenticated by Miss. Rasika S Kapale, HOD, Department of Botany, Smt. V G Degree college for womens, Kalaburagi. A voucher specimen is preserved in the herbarium of Department of Botany (Voucher No.1006).

Preparation of plant Extracts:

Extraction Procedure:

The drug will be subjected to systematic phytochemical screening by ethanolic extract and subjected to phytochemical investigation by qualitative chemical analysis.

Preparation of Plant Extracts:[8]

Selected crude drugs will be collected and shade dried, powdered in a mechanical grinder and passed through a sieve no.40 to obtain powder of desired particle size The powdered material will be subjected to ethanolic extraction in soxhlet apparatus. The filtrate will be collected and evaporated to dryness under reduced pressure using a Rotary flash evaporator. The extract obtained will be weighed; its percentage will be calculated in terms of air-dried weight of plant material and tabulated. The dried extract will be stored in dry sterilized small containers at 4˚C until further use.

III. PRELIMINARY PHYTOCHEMICAL STUDIES

A known quantity of dried ethanolic polyherbal extract of *Semecarpus anacardium*, *Vachellia nilotica*, and *Portulaca quadrifida* is followed for qualitative tests i.e.,

1. Test for carbohydrates:

To 2 ml of test solution adds two drops of the Molish reagent (a solution of α napthol in 95% ethanol). The solution is then poured slowly in to a test tube containing 2 ml of conc. Sulphuric acids so that two layers form. The formation of a purple product at the interface of the two layers indicates the presence of carbohydrate.

2. Test for proteins:

It is used to determine the presence of peptide bonds in protein. To 3 ml of test sample is add 3% NaOH and a few drops of 1% $CuSO_4$. The solution turns from blue to violet (purple) or to pink. That indicates the presence of protein.

3. Test for starch:

Mix 3 ml of test solution and few drops of dilute iodine solution. Blue color appears. It disappears on cooling and reappears on heating.

4. Test for amino acids:

To 5ml of test sample solution is add a few drop of 40% NaOH and 10% of lead acetate and boiled the solution formation of black precipitate show the presence of amino acids.

5. Test for steroids:

To 2 ml of extract and add 2 ml of chloroform and add 2 ml conc. Sulphuric acid. Shake well, chloroform one layer appear and acid layer show greenish yellow florescence which indicate the presence of steroids.

6. Test for glycosides:

To the solution of extract add glacial acetic acid, few drops 5% ferric chloride and concentrated sulphuric acid are added, and observed for a reddish-brown coloration at the junction of two layers and bluish green color in upper which indicates presence of glycosides.

7. Test for flavonoids:

To 2 ml of extract and few drops of 1% of Ammonia solution. A yellow coloration is observed for the presence of flavonoids.

8. Test for alkaloids:

To 0.5g of each extracts adds 5ml of 1% of aqueous hydrochloric acid and kept in water bath: 1ml of the filtrate is to be treated with Mayer's reagent (Potassium iodide). Formation of a yellow-colored precipitate indicates the presence of alkaloids.

9. Test for tannins:

To 0.5ml of extract solution, 1 ml of water and 1-2 drops of ferric chloride solution was added. Blue color was observed for gallic tannins and black color for catecholic tannins.

10. Test for saponins:

To 1 ml of extract solution, 1 ml of water and shake it. Persistent foam indicates presence of saponins.

11. Test for terpenoids:

2ml extract was mixed with 2ml of chloroform in a test tube. To this 3ml of conc. Sulphuric acid was added along the walls of the tube to form a layer. An interface with a reddish-brown coloration confirmed the presence of terpenoids.

12. Test for gums:

To 1 ml of extract add 3 ml of Dil. Hcl solution is added drop by drop till red coloration visualizes the presence of gums.

IV. TEST ANIMALS

For the study, adult albino Wistar rats aged between 8-14 weeks and weighing 150-155 grams were allowed to acclimatize for a period of 7 days before the beginning of the experiment. They were housed in groups of seven in polypropylene cages with soft wood shavings as bedding, which were renewed every 24 hours. The rats were maintained under 12/12 hours light/dark cycles, with a relative humidity of 50-60% and a temperature of 22±3°C. They were provided with rat pellet diet (Gold Moher, Lipton India Ltd) and water ad libitum on a regular basis.

Induction of diabetes:[10]

Diabetes was induced in overnight fasted rat by a single intraperitoneal injection of Alloxan (80 mg/kg body weight) in a 0.1 M sodium citrate buffer (pH 4.5). The age-matched control rat received an equivalent amount of citrate buffer. Food and water intake were closely monitored daily after alloxan administration. The www.wjpr.net Vol 8, Issue 10, 2019. 206 Islam et al. World Journal of Pharmaceutical Research development of hyperglycemia in rat was confirmed by fasting (16 hour) blood glucose measurement in the tail vein blood, 48 hours after Alloxan administration, with a portable glucometer (Accu-Chek, Roche, Germany). The animals with fasting blood glucose level ≥ 11.0 mmol/L with other symptoms of diabetes mellitus such as polyphagia, polydipsia, polyuria, and weight loss were considered as diabetic and included in the study.

Experimental groups:

After one-week acclimatization period, the animals were divided into five groups with five animals in each. The rat grouping was as follow:

Group-1 (Normal control): Rat feed with standard pellet diet and water.

Group-2 (Diabetic control): Diabetic rat without treatment.

Group-3 (Positive control): Diabetic rat were treated by Glibenclamide at dose of 5 mg/kg body weight.

Group-4 (Treated-1): The diabetic rat treated with Petroleum Ether extract of poly herbal (PEEP) formulation at a dose of 100 mg/kg body weight for 21 days.

Group-5 (Treated-2): The diabetic rat treated with Petroleum Ether extract of selected polyherbal (PEEP) at a dose of 200 mg/kg body weight for 21 days.

Group-6 (Treated-1): The diabetic rat treated with ethanol extract of polyherbal (EEP) at a dose of 100 mg/kg body weight for 21 days.

Group-7 (Treated-2): The diabetic rat treated with ethanol extract of Polyherbal (EEP) at a dose of 200 mg/kg body weight for 21 days.

Method of Collection of Blood:

Blood was collected from the orbital sinus with the help of a capillary tube by pressing the thumb behind the angle of the jaw resulting in the engorgement of the retro orbital plexus (Figure-2). The serum of each animal was estimated for different biochemical parameters. All groups were covered in the study.

Collection of Blood

Determination of Serum glucose

Fasting serum glucose was estimated by the oxidase method.[11] Determination of serum total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol and total cholesterol/HDL cholesterol (T.C/HDL C.). Serum was separated and analysed for serum total cholesterol, triglycerides, HDL cholesterol.[12]

Measurement of biochemical parameters:

Blood glucose level was measured by glucose oxidase peroxidase method. Plasma concentrations of triglyceride (TG), total cholesterol (TC), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), VLDL, CRP, SGPT, SGOT, were measured using a quantification kit (Linear chemicals, Barcelona, Spain) by automatic Bio-analyzer (Hitachi 7180, Hitachi, Tokyo, Japan).

Statistical analysis.

Data was expressed as means ± S.E.M. Statistical analysis was made by one-way ANOVA and post hoc Dunnet test, with $P < 0.05$ considered as significantly different.

V. RESULTS

Result describes Phytochemical screening of polyherbal formulation.

Sl.no	Phytochemical constituents	Petroleum ether extract	Ethanol extract
01	Alkaloids	+	+++
02	Flavonoid	++	++
03	Tannin	+	+
04	Terpenoid	-	-
05	Glycoside	-	+
06	Saponin	++	++
07	Carbohydrates	+	+
08	Proteins	+	+
09	Amino acids	+	+
10	Steroid	++	++
11	Gums	-	-

Effects of Petroleum ether extract polyherbal (PEEP) and Effects of ethanolic extract polyherbal (EEP) on blood glucose level:

In in vivo antidiabetic assay, PEEP and EEP were administered to evaluate the blood glucose level of rats.

The plant extract produced significant changes in the blood glucose level in Alloxan-induced diabetic rat.

The administration of plant extract showed significantly $p<0.01$ to $p<0.001$) reduction of blood glucose level in both concentrations of 100 mg/kg and 200 mg/kg body weight respectively compared to diabetic control rat.

This level of reduction was very close to positive control rat (here 5mg/kg body weight Glibenclamide is used as standard).

In 5th to 21st days, PEEP at both doses (100 mg/kg and 200 mg/kg body weight) lowered the blood glucose level by 1.09% - 58.85% and 6.88% - 68.40% and EEP at both dose (100mg/kg and 200 mg/kg body weight) lowered the blood glucose level by 8.19% - 52.70% and 12.69% -61.19% respectively.

Effects of PEEP and EEP on lipid profile level:

Fig. 1 showed the serum lipid profile levels of Total cholesterol (TC), Triglycerides (TG), LDL, VLDL, HDL and hypercholesterol of control and Alloxan-induced diabetic rat.

All lipid except HDL parameters showed in significantly different at $p<0.001$. Reduction of Total Cholesterol (TC) level was 21.54 % (in 100 mg/kg body weight) and 31.39 % (in 200 mg/kg body

weight) observed in Petroleum ether extract of selected polyherbal PEEP and 17.63% (in 100 mg/kg body weight) and 24.26 % (in 200mg/kg body weight) observed in ethanol extract of polyherbal (EEP) treated diabetic rat group whereas in positive control group reduction was 40.79 %. In 21 days observation the treatment groups showed significant decrease ($p<0.001$) of total cholesterol compared with the diabetic control group.
The Levels of triglyceride in the diabetic group were increased after 21 days of alloxan induction. Serum triglyceride of treatment group of rat was lower than that of diabetic control.
In Fig.1 the diabetic control group showed an increase in LDL levels higher than the (normal) control group. LDL level was significantly reduced ($p<0.001$) with Polyherbal treatment at 41.09 % (in 100 mg/kg body weight of PCA), 79.39 % (in 200 mg/kg body weight of PEEP) and 23.68 % (in 100mg/kg body weight of EEP) and 41.27 % (in 200mg/kg body weight of EEP) respectively.
VLDL level was significantly reduced ($p<0.001$) with Polyherbal treatment at 23.29 % (in 100 mg/kg body weight of PEEP), 33.13 % (in 200 mg/kg body weight of PEEP) and 19.08 % (in 100mg/kg body weight of EEP), 28.6% (in 200mg/kg body weight of EEP) respectively.
Whereas the HDL level increased by 20.16% (in 100 mg/kg body weight of PEEP), 44.7% (in 200mg/kg body weight of PEEP) and 21.96% (in 100mg/kg body weight of EEP) and 36.66 % (in 200 mg/kg body weight of EEP) respectively.
Glibenclamide (5 mg/kg body weight) treated rat showed the reduction of TG by 39.55 %, LDL by 78.54%, VLDL by 35.77% and an increase in HDL by 40.11% respectively.

Effects of PEEP and EEP on serum SGPT, SGOT and CRP level:
Serum SGPT and SGOT levels were increased significantly ($p<0.001$ to $p<0.01$) in diabetic rat compared to the normal rat and these were also compensated considerably ($p<0.001$) by the oral administration of PEEP, EEP and glibenclamide (Fig. 2).
The percent of lowering of SGPT level by Polyherbal both Petroleum ether and ethanol extract from diabetic control groups were 13.81 % to 25.50 % and 11.11% to 19.01% at dose 100 mg/kg and 200 mg/kg body weight respectively whereas 35.49 % for glibenclamide.
The reduction of SGOT level was highly significant ($p<0.001$) for Polyherbal both petroleum ether and ethanol extract at 15.10 % to 31.33 % and 9.00 % to 24.30% at dose 100 mg/kg and 200 mg/kg body weight respectively, whereas 30.50 % for glibenclamide. CRP is a potent marker of hepatic and cardiovascular diseases, which is increased in diabetic condition.
The administration of PEEP, EEP and glibenclamide reduced the CRP level significantly ($p<0.001$ to $p<0.01$) compared to the diabetic control rat, MCUF administration at 100 and 200mg/kg body weight doses lowered the CRP level by 32.99% and 40.10% respectively.

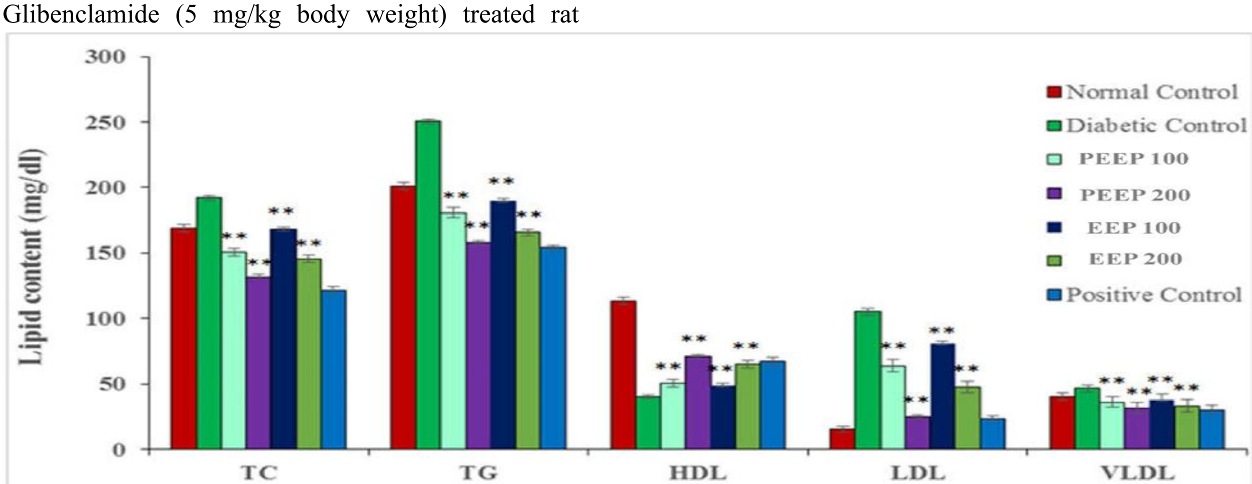

Figure 01: Effects of Petroleum ether extract and ethanol extract of Polyherbal on lipid profile of diabetic rat after 21 days treatment.

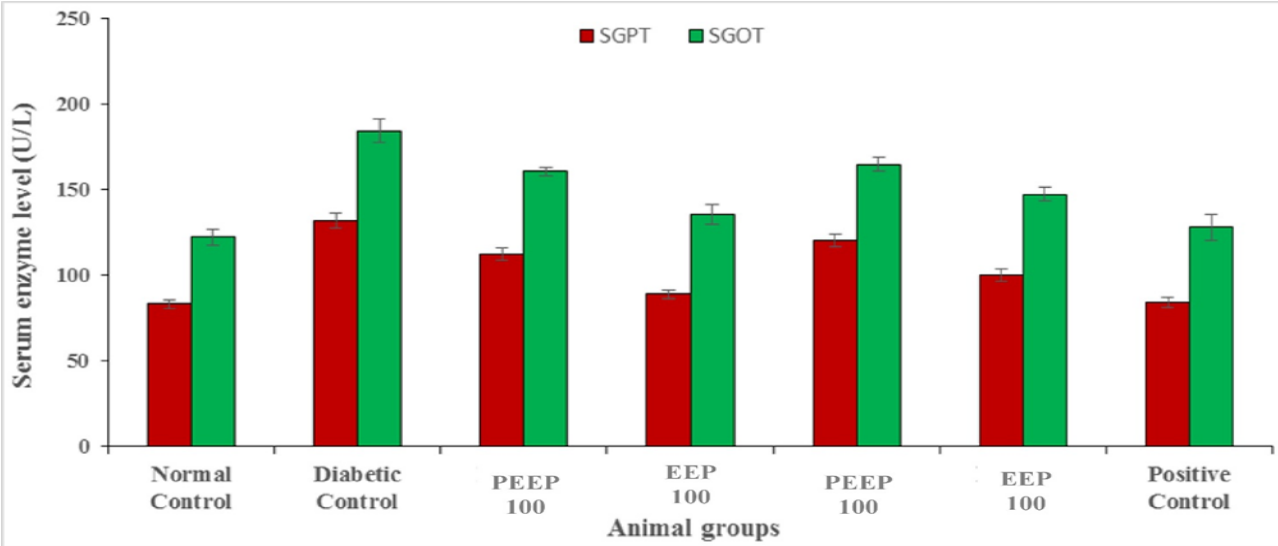

Figure 02: Effects of Petroleum ether (PEEP) and ethanol extract (EEP) of polyherbal on serum SGPT and SGOT level in diabetic rat after 21 days treatment.

Effects of Petroleum ether extract and ethanol extract of Polyherbal on blood glucose level in Alloxan-induced diabetic rat.

Groups of animals		Serum glucose concentration (mmol/L)				
		Day 1	Day 5	Day 10	Day 15	Day 21
NORMAL CONTROL		5.99±3.01	5.60±4.40	6.01±1.69	6.91±2.01	5.9±4.30
DIABETIC CONTROL		19.2±4.56[a]	19.2±4.02[a]	21.32±4.10[a]	23.9±3.18[a]	27.9±8.01[a]
POLYHERBAL		20.2±6.99	16.0±4.01[b]	12.1±3.33[c]	11.90±3.30[c]	10.1±3.01[c]
Treatment	PEEP 100	21.7±1.77	19.9±1.76[b]	15.0±8.61[b]	14.9±3.67[b]	13.6±8.96[c]
	PEEP 200	21.3±0.92	18.8±3.59[b]	15.7±0.53[b]	11.5±2.20[c]	11.3±2.06[c]
	EEP 100	23.1±0.68	21.3±0.51[b]	15.5±1.10[b]	17.3±5.52[b]	14.4±6.37[c]
	EEP 200	27.3±2.07	24.5±2.09[b]	21.3±5.73[b]	19.6±2.67[c]	12.4[b]±5.17[c]

All values are expressed as mean ± standard deviation (n=6). [a]$p<0.001$compared with general control group; [b]$p<0.01$ and [c]$p<0.001$ compared with diabetic control group.

VI. DISCUSSION

Our results demonstrated that TG, TC, VLDL, LDL levels decreased in diabetic rat with the administration of polyherbal (PEEP & EEP) extract. The hyperlipidemia associated with diabetes may result from an accelerated hepatic triglyceride biosynthesis and the release of VLDL without an increase in its rate of clearance from the blood by lipoprotein lipase, which is dependent on the insulin/glucagon ratio. Our current study clearly indicated that the oral administration of PEEP and EEP have significantly ($p<0.01$ to $p<0.001$) antidiabetic and antihyperlipidemic ($p<0.001$) activities, which are quite similar to the antidiabetic and hypolipidemic activities of glibenclamide in alloxan induced diabetic rat compare to the diabetic control rat.

VII. CONCLUSION

This preliminary study confirms the hypoglycemic effect of Polyherbal (PEEP & EEP) together with other beneficial effects in diabetic rat. These results suggest that the petroleum ether extract and ethanol extract of Polyherbal (PEEP & EEP) may improve the metabolic disruption produced by diabetes. However, further research is needed to gain a better understanding of its potential therapeutic action, the implicated phytochemical constituents and the exact mechanism of action.

VIII. ETHICAL CLEARANCE

This research work was approved by the Institutional Animal, Medical Ethics, Bio-Safety and Bio-Security Committee (IAMEBBC) for Experimentations on Animal, Human, Microbes, and Living Natural Sources, memo no. 2348/PO/Re/S/2025/CCSEA. Institute, MAM College of Pharmacy, Kalaburagi, Karnataka – India.

REFERENCES

[1] Poznyak. A et al. The diabetes mellitus–atherosclerosis connection: The role of lipid and glucose metabolism and chronic inflammation. International Journal of Molecular Science. 2020; 21 (5). Page 1835.

[2] Rossi. M.C et al. Impact of severe and symptomatic hypoglycemia on quality of life and fear of hypoglycemia in type 1 and type 2 diabetes. Results of the Hypos-1 observational study. Nutrition, Metabolism and Cardiovascular diseases. 2019; 29 (7): Page 736-743.

[3] Davies MJ, D'Alessio DA, Fradkin J, Kernan WN, Mathieu C, Mingrone G, Rossing P, Tsapas A, Wexler DJ, Buse JB. Management of Hyperglycemia in Type 2 Diabetes, 2018. A Consensus Report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). Diabetes Care 2018; 41: Page 2669-2701.

[4] World Health Organization. Definition, Diagnosis and Classification of Diabetes Mellitus and Its Complications. Department of Non communicable Disease Surveillance; Geneva, Switzerland: 1999. [Google Scholar].

[5] American Diabetes Association 2. Classification and diagnosis of diabetes: Standards of Medical Care in Diabetes–2019. Diabetes Care. 2019; 42(Suppl. 1): S13–S28.

[6] Goodman ls, Gilman A. The Pharmacological Basis of Therapeutics. 2nd ed. New York: Macmilan Company; 1955. pp. 1636–8. [Google Scholar].

[7] Rerup CC. Drugs producing diabetes through damage of the insulin secreting cells. Pharmacol Rev. 1970; 22: 485–518.

[8] Khasim. SM and Dr. Kumar. EP Antimicrobial activity and in vitro anticarcinogenic properties of portulaca quadrifida linn'on colon cancer using different extracts. World Journal of Pharmaceutical Research. 2015; 4(11): 1644-1652.

[9] Kokate, C. K. Practical Pharmacognosy. 4th Edn. Vallabh Prakashan, New Delhi. 1993; Pp.107-111, 178-181.

[10] Islam MS et al.Experimental rodent models of type 2 diabetes: a review. Expimental and Clinical Pharmacology. 2009; 31(4): Page 249-261.

[11] Dingeon B, Ferry JP, Automatic assay of blood sugar by Trinder's method. Biol Clin (Paris). 1975; 33(1): Page 3-13.

[12] Shawar SM, et al. Hypercholesterolemia among apparently healthy university students. Oman Med Journal. 2012; 27(4): 274-280.