Contents lists available at UGC-CARE

International Journal of Pharmaceutical Sciences and Drug Research

[ISSN: 0975-248X; CODEN (USA): IJPSPP]

Available online at www.ijpsdronline.com

Research Article

Effect of *Vitex trifolia* on Alloxan induced Diabetic Rat

Harsha M. Naikwad, Neelakanth M. Jeedi*

Department of Pharmacology, K L E College of Pharmacy, Vidyanagar, Hubballi-580031, (A constituent unit of KLE Academy of Higher Education and Research, Belagavi) Karnataka, India

ARTICLE INFO

Article history:

Received: 05 June, 2020

Revised: 15 December, 2020

Accepted: 30 December, 2020

Published: 30 January, 2021

Keywords:

Alloxan,
Anti-diabetic,
Vitex trifolia.

DOI:

10.25004/IJPSDR.2021.130102

ABSTRACT

The leaves of plant *Vitex trifolia* by tradition used to treat of diabetes and correlated complications. Plants *V. trifolia* and *Vitex negundo* belongs to the Verbenaceae family, have some similar phytoconstituents. Leaf extract of *Vitex negundo* proven for its anti-diabetic property. Diterpenoids present in fruits of *V. trifolia,* they have anti-diabetic property. Vitexilactone a phytoconstituents in *V. trifolia* has insulin sensitizing property. The present study aimed to investigate in *vivo* anti-diabetic activity of aqueous leaf extract of *V. trifolia* in alloxanized diabetic albino wistar rats. Diabetes induced in rats by injecting alloxan hydrate at dose 150 mg/kg body weight. Diabetic rats were treated with 100 and 200 mg/kg of aqueous extract of V. trifolia for 21 days. Anti-diabetic activity of extract assessed by measuring blood glucose by Trinder's method, insulin by enzyme-linked immunosorbent assay, lipid profile by colorimetric methods, total proteins by Biuret method and calcium measured by using ligand Arsenazo III in an aqueous alkaline medium. Aqueous extract of *V. trifolia* did not show significant changes of blood sugar level in normal rats. But extract significantly reduces blood sugar, total serum cholesterol, triglycerides levels but increases insulin, HDL-cholesterol, total proteins, and calcium in diabetic rats. Aqueous extract of *V. trifolia* showed significant anti-diabetic activity in alloxan-induced diabetic rats.

Introduction

Diabetes mellitus (DM) is due to the disorganization of metabolism, characterized by high blood glucose levels. A metabolic disorder is as a result of absolute shortage or lack of insulin to act on target tissues.[1] DM has two categories: Type 1 in this insufficiency of insulin because of autoimmune antibodies damaging the pancreas' beta cells. In Type 2 normal insulin level but tissues developed resistance to it or less insulin secretion. Normally Type 2 individuals are habitually overweight.[2] High glucose levels in the blood for a long duration that causes small and large blood vessel abnormalities. The abnormality in lipid metabolism leads to hyperlipidemia, it is marked by high cholesterol, triglycerides and altered lipoprotein levels.[3] These abnormalities are the main cause for illness and death in DM. Africa's-International Diabetes Federation reports more than 1.4 crore people suffering with DM in their country, and they predicted the number of patient rise to 2.8 crores by the year 2040. That attributed significantly to the global health burden and a major contributor for the global health afflicting 285 million adult populations and expected to accelerate to 439 million by 2030. Annually, about 1.5 million deaths were reported worldwide due to diabetes; as per World Health Organization (WHO) report in India alone nearly about 31 million people suffered with diabetes in the year 2000. In the future, it may to grow up to 79 million by 2030.[4] Oral hypoglycemic agents used to treat DM causes different side effects like hematological effects and affects the functions of vital organs like the liver and kidney. In addition, there is no perpetual cure for diabetic neuropathy. However, symptomatic treatments have shown limited success. Worldwide nowadays, a number of medicinal plants have been reported and are useful for treating DM. WHO suggests using traditional plants for treating DM, therapeutically effective, nontoxic, with little or no unwanted effects.[5]

***Corresponding Author:** Dr. Neelakanth M. Jeedi

Address: Assistant Professor, Department of Pharmacology, K. L. E. College of Pharmacy, Vidyanagar, Hubballi-580031, Karnataka, India.

Email ✉**:** neelakant_jeedi@rediffmail.com

Tel.: +91-8951370708

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Form of ancient times, many traditional plants were used to cure various diseases, including diabetes. The plants are important and the main sources of drugs to treat various illnesses, particularly in countries on development like India. Most population don't have resources or unable to access facilities.[6] But in the 21st century, natural source medicines are popular in both underdeveloped and developed countries because of free availability or less price and less side effects. Therapeutically important or active chemicals in plants like tannins, flavonoids, alkaloids, proteins, phenolic compounds, and saponins acquire glucose-lowering actions.[7] Animals can be made diabetic by injecting Alloxan or Streptozotocin (STZ), which resembles natural DM. STZ damages pancreatic beta cells by producing free radicals and acting on chromosomes. This causes suppression of insulin production and its release.[8] Chemically alloxan is Pyrimidine-2,4,5,6(1H,3H)-tetraone hydrate. Alloxan causes lipid peroxidation and breaks DNA. It causes high blood glucose level and glycosuria in several species of animals.

The plant *V. trifolia* (VT) is an aromatic coastal deciduous shrub grown India, Bangladesh, and Sri Lanka. It is used as a traditional folk medicine for various ailments and was evaluated for *in vitro* antimicrobial, antioxidant, *in vivo* hepatoprotective, anti-nociceptive, anti-asthmatic, anti-tubercular, and analgesic activity—many herbs used for diabetes in folk medicine. Plants used in the traditional system of medicine have little or no side effects.[9] The plants contain polyphenolic compounds, flavonoids, proteins, tannins, phytosterols and saponins reported for anti-diabetic activity. The use of medicinal plants and their phytochemicals for treating diabetes is research for safer alternatives to presently used anti-diabetic drugs. The leaves of plant *V. trifolia* by tradition are used to treat diabetes and correlated complications.[10,11] Plants *V. trifolia* and *V. negundo* belong to the Verbenaceae family, with some similar phytoconstituents like carbohydrates and alkaloids saponins, flavonoids, phenols, tannins, and terpenoids in their leaves.[12] Leaf extract of *V. negundo* proven for its anti-diabetic activity.[13] Diterpenoids present in fruits of *Vitex trifolia*, [14] diterpenoids proven to have anti-diabetic property.[15] The ethyl acetate extract of *V. trifolia* contains Vitexilactone, a phytoconstituent useful as an anti-diabetic agent due to its insulin-sensitizing action selective agonist for peroxisome proliferator-activated receptor γ (PPARγ).[16] With this background, the present study aims to evaluate the effect of aqueous *V. trifolia* leaves on alloxan elicited diabetic rats.

Materials and Methods

Collection of Plant Materials: Leaves of *V. trifolia* collected at KLE College of pharmacy, botanical garden, (Hubballi, Karnataka), authenticated by Head Dept. of Botany, SK. Arts and HSK. Science College, Vidyanagar, Hubballi. The leaves were cleaned with tap water and dried for thirty days at room temperature, coarse powdered and preserved for further processing.

Plant Extract Preparation: Cold maceration method followed to prepare aqueous extract. The coarse 100 grams of dried, coarse powder weighed, macerated with 100 ml water for 6 hours, shaken frequently and kept for 18 hours, not losing any solvent. Transfer to plate and evaporate to dry in a water bath.

Preliminary Phytochemical Screening: Phytochemical investigations is performed to identify the occurrence of steroids, carbohydrates, proteins, amino acids, triterpenoids, alkaloids, tannins, flavonoids and a phenolic class of compounds aqueous extract of *V. trifolia* leaves.[17]

Animals: Albino Wistar rats (150-250gm) of both sex, housed in clean cages and maintained under the natural light & dark. Standardized laboratory food and water *ad libitum* given to animals. Animals acclimatized for one week before experiment. Rats randomly selected for different experimental groups after seven days of acclimatization. The experimental protocols were reviewed and approved by IAEC.

Chemicals: Alloxan hydrate, commercial diagnostic kits required to estimate biochemical like lipid profile, blood glucose, insulin, enzymes level etc., purchased from local vendors of Erba Diagnostic Pvt. Ltd. The drug MFM procured from KLE College of Pharmacy, Hubballi, Karnataka, India. Chemicals of analytical grade purchased by local vendors at Hubballi, Karnataka.

Acute Toxicity Study

Acute toxicity tests for aqueous extract of *V. trifolia* leaves performed on albino mice weighing between 25-30 gm. The step-wise up-down method was selected for toxicity studies. Female mice were starved full night and randomly separated in six groups (n = 3) they were orally treated with aqueous extracts suspension in dose levels 100 mg/kg, 500 mg/kg, 1 gm/kg and 2 gm/kg body weight by using a stomach tube. The animals were individually observed at least once during 30 minutes to indicate compound-related toxicity and mortality, periodically for the first 24 hours, with special attention for 4 hours.

Induction of Diabetes

Diabetic condition practically made in the rats of groups 2–5. The overnight fasted rats injected alloxan hydrate (150 mg/kg) dissolved in normal saline by subcutaneous route. After 3 days, induction is confirmed by measuring blood glucose, if it is above 250mg/dl considered diabetic and taken for study.[18]

Experimental Design

Individually weighted animals randomly allotted to five groups, animals six in each group. Group one non-diabetic and the remaining four diabetic groups receive various treatments as mentioned below. An equal number of male and

females were maintained in each group and caged separately (6 rats per cages) throughout the experimental period.

Groups

Group 1: Control group
Group 2: Alloxan induced diabetic group
Group 3: Alloxan induced diabetic treated with *V. trifolia* leaves extract (100mg/kg)
Group 4: Alloxan induced diabetic treated with *V. trifolia* leaves extract (200mg/kg)
Group 5: Alloxan induced diabetic treated with (standard drug) MFM (100mg/kg).

The vehicle, MFM and extract solutions were administered to the respective group of animals by oral gavages, one time in a day for three weeks. Rats have fasted for 16 hours; blood collected retro-orbitally using capillary tube under mild ether anesthesia. Blood allowed clots for 10 minutes, serum alienated after 10 minutes centrifuging at 5000 rpm and used for study. To assess anti-diabetic activity, various biochemical parameters measured using blood. The animals streamlined after cervical dislocation on the twenty-first day. Blood glucose estimated in all groups, before and after treatment on 1st, 3rd, 7th, 14th, and 21st day after the respective treatment. At the end of 21 days of treatment period, the rats were subjected to overnight fasting; blood sample collected and analyzed the glucose, insulin, total cholesterol (TC), total protein, triglycerides (TG), high-density lipoprotein (HDL), and calcium in all the groups.[19-25] In addition to the above, bodyweight also measured. Blood glucose was measured by Trinder's method.[26] Insulin measured by enzyme-linked immunosorbent assay. TC measured by enzymatic colorimetric (chod-pap) method.[27] TG estimated by the method of McGowan et al. and Fossati et al.[28,29] HDL-cholesterol by enzymatic colorimetric method.[30] Total protein measured by Biuret Method. [31] Serum calcium was measured by using ligand Arsenazo III in an aqueous alkaline medium.[32] All these biochemical measured using a standard kit from ERBA and semi-Autoanalyser.

Statistical Analysis

The data obtained furnished as the Mean ± SEM (standard error of mean). Statistical analysis was done by one-way analysis of variance (ANOVA), subsequently by Tukey's multiple evaluation tests. p-values expressed significance, and if p-value is <0.05, the results were regarded as statistically significant.

Results

Plant Extract: The percentage yield of coarsely powdered leaves aqueous extract of *V. trifolia* obtained by cold maceration processes is 8.23%. The physical property of extract – sticky, brownish-black.

Preliminary Phytochemical Screening: The aqueous extract of leaves of *V. trifolia* tested qualitatively, that revealed the presence of carbohydrates, flavonoids, steroids, triterpenoids, proteins, amino acids, tannins, and saponins.

Acute Toxicity (LD50) Study

Acute oral toxicity study, No death or severe side effects observed due to aqueous extract of leaves of *V. trifolia* at a dose of 2000 mg/kg. 2000 mg/kg is considered the limit dose. Therefore, 1/10 and 1/20 of the extract dose (100 and 200 mg/kg) were used as effective doses in this study.

Effect of Extract on Body Weight of Rats

Slight increase in normal control group animals seen, it is due to normal growth. Diabetic animals lost their body weight considerably (p <0.001) compared to normal control due to the alloxan effect. Further, the diabetic animals kept losing their body weight during the experiment because of diabetic animals not being treated. A slight increase in body weight was witnessed (p <0.01) in the animals treated with MFM (100mg/kg). VT (100 and 200 mg/kg) treatment normalizes animals' body weight by extinguishing the effect of alloxan. However, there was much improvement in animals' body weight seen on 3rd week of VT and MFM treatment. Results are shown in Table 1.

Effect of VT on Blood Glucose in Alloxan-persuaded Diabetic Rats

VT extract was given at doses of 100 and 200 mg/kg for three weeks to treat diabetes. It was observed that a significant increase (p <0.001) in the serum glucose level of diabetic compared to the non-diabetic animals further increases in glucose on 7th, 14th, and 21st day. MFM reduces

Table 1: Effect of VT on Body Weight (g) in Experimental Rats

Treatment	*Control*	*Diabetic control*	*VT 100 mg/kg*	*VT 200 mg/kg*	*MFM 100 mg/kg*
Before treatment	180.2	180.210.04	192.212.242	200.152.151	182.452.31
After treatment 1st day	185.232.10	178.274.44	181.022.319	185.164.757	170.213.33
3rd day	189.231.70	162.13.868	177.145.346	180.233.127	175.232.21
7th day	195.343.33	151.243.59*	180.252.149*	188.241.400*	181.112.50*
14th day	201.142.40	142.413.18	183.451.400**	192.534.254***	185.413.14***
21st day	204.532.35	130.152.257	188.224.264***	195.2664.015***	188.213.25***

Values presented as mean SEM (n = 6). VT-*Vitex trifolia*, MFM-Metformin, p <0.05*, p <0.01**, p<0.001***, compared with mean values before treatment of respective groups.

Table 2: Effect of VT on Blood Glucose level (mg/dl) in experimental rats

Treatment	*Control*	*Diabetic control*	*VT 100 mg/kg*	*VT 200 mg/kg*	*MFM 100 mg/kg*
Before treatment	79 ± 0.21	83 ± 1.35	81 ± 2.08	78 ± 0.21	82 ± 1.24
After treatment 1st day	82 ± 1.04	312 ± 0.49***	315 ± 1.34*	310 ± 0.38*	317 ± 1.51**
3rd day	85 ± 0.43	340 ± 0.63***	292 ± 0.57*	274 ± 1.57*	293 ± 0.68**
7th day	81 ± 0.65	398 ± 0.76***	250 ± 0.69*	220 ± 0.69*	205 ± 0.81**
14th day	83 ± 0.51	415 ± 0.58***	212 ± 0.47*	185 ± 0.24*	141 ± 0.97**
21st day	85 ± 0.87	430 ± 0.87***	165 ± 0.96*	142 ± 1.51**	92 ± 1.25**

Values presented as mean SEM (n=6). VT-*Vitex trifolia*, MFM-Metformin, $p<0.05$*, $p<0.01$**, $p<0.001$***, compared with mean values before treatment of respective groups.

Table 3: Effects of VT extract on lipid profile, total protein, calcium and insulin levels in experimental rats

Group	*Control*	*Diabetic control*	*VT 100 mg/kg*	*VT 200 mg/kg*	*MFM 100 mg/kg*
Total Cholesterol (mg/dl)	87.781.462	149.573.354***	112.054.150*	93.312.039**	83.942.068***
Triglycerides (mg/dl)	88.321.633	124.503.317***	112.452.97	103.933.497***	93.023.157***
HDL Cholesterol (mg/dl)	25.883.085	16.732.369***	19.312.434	22.562.256**	29.341.837***
Total protein (g/dl)	8.6370.703	3.800.439***	6.410.353	7.480.188**	8.720.958***
Calcium (g/dl)	10.560.444	5.970.543***	8.110.708	9.060.752**	9.490.543***
Serum Insulin µIU/mL	9.240.385	5.060.718***	6.320.425*	8.140.927**	8.790.654***

Values presented as mean SEM (n = 6). VT-*Vitex trifolia*, MFM-Metformin, $p<0.05$*, $p<0.01$**, $p<0.001$***, compared with mean values before treatment of respective groups.

Table 4: Effect of VT extract on Blood glucose (mg/dL) in normal rats

Treatment	*Control*	*VT 100 mg/kg*	*VT 200 mg/kg*
Before administration	84.21.355	87.521.078	85.331.601
After administration 30 min	84.61.30	86.131.29	83.431.957
60 min	83.721.88	85.161.885	81.621.901
120 min	84.30.745	83.40.745	79.581.105

Values presented as mean SEM (n = 6). VT-*Vitex trifolia*, $p<0.05$*, $p<0.01$**, $p<0.001$***, compared with mean values before treatment of respective groups.

(p <0.001) levels of glucose consistently compared to diabetic animals. Extract treated group animals; VT 100 and 200 mg/kg reduces (p < 0.01) serum glucose steadily on 7th, 14th and 21st day related to diabetes. The VT extract reduces blood glucose levels dose-dependently. On 21st day VT 200 mg/kg treated group animal's has blood glucose nearer to normal. However, no substantial difference was observed between the VT 200 mg/kg treated and MFM treated animals. VT extract exhibited an ideal lowering effect on glucose at 200 mg/kg. Results are shown in Table 2.

Effect of VT Extract on Lipid Profile in Diabetic Rats

Untreated hyperglycemic rats showed high TC, TG but low calcium, insulin, and HDL-cholesterol significantly related to normal rats. VT extract treatment displayed a significant lowering effect on TC and TG but improved the calcium, insulin, and HDL-cholesterol significantly related to diabetic rats. The high dose of extract effect is comparable to standard MFM. Normal calcium due to extracting effect may be liable for the secretion of normal quantity of insulin by the pancreas. [33] Results are shown in Table 3.

Effect of VT Extract on Blood Glucose (mg/dL) in Normal Rats

The effect of VT extract on blood glucose levels of normoglycemic rats tested apart from the diabetic rats. The findings say that there are no significant changes observed in normal group animals. However, there is a slight decrease in blood glucose levels observed after one and two hours of administration of VT extract 100 and 200 mg/kg. The changes in blood glucose with extract are not significant. Results are shown in Table 4.

Discussion

DM occupies now one of the main causes of serious malady in 21 Century. The cause for DM is a lifestyle, genetic etc. Biochemical variations occur mainly in carbohydrate, protein and lipid metabolism. A change in protein levels,

lipids, glucose, insulin and elements like calcium occurs. This entire biochemical change occurs because of altered structure and function of the pancreas, enzyme activity, and resistance development to hormones. A high TC and TG attributed to stop cholesterol catabolism, lack of insulin or adipose tissue releases fatty acids by lipolysis. The rise in HDL-cholesterol level used to wash out the excess cholesterol from the body is considerably reduced in DM. The improvement in HDL-cholesterol is complemented by an improved breakdown of VLDL and placing of TG in the central of HDL with cholesterol. This lipid normalizing action of VT extract may be due to its actions on enzymes involved in lipid metabolism. This contributes to prevent the development of neuropathy, atherosclerosis and cardiovascular complications associated with diabetes. Abnormalities in proteins glycation non-enzymatically also atherogenic, excessive catabolism of protein further contributes in microvascular complications. VT extract prevents the abnormal breakdown of proteins and normalizes total protein. Calcium is an element essential for homeostasis. Calcium levels in the body regulated by hormones by acting on renal, digestive and skeletal systems. If changes in blood calcium levels affect insulin release by the pancreas, glucose sensitivity, bones, and blood pressure.

The rise in oxidative stress and its squealed are widely accepted in diabetes. Several studies have drawn attention to antioxidants may be a good strategy to reduce diabetes also related complications. An evaluation of alloxan action, a pancreatic beta-cell cytotoxin has shown physiological and biochemical disorder leads to the diabetic state. Alloxan (150 mg/kg) demonstrated hyperglycemia due to metabolic stress, altered insulin secretion, and due to progressive oxidative stress. Twenty-one days of treatment of extract corrected the protein, lipid, and carbohydrate metabolism. It almost normalizes the insulin, total protein, calcium, and lipid profile by normalizing the pancreas' structure and functions. This possible mechanism is supported by elevated levels of insulin in diabetic animals treated with VT extract. Flavonoids are natural antioxidants present in the extract also contribute to the anti-diabetic activity. The flavonoids, steroids, tannins, proteins, saponins and phenolic compounds present in aqueous extract of VT leave compounds are responsible for the anti-diabetic activity.

Conclusion

This study revealed that V. trifolia leaves' aqueous extract has significant anti-diabetic activity in alloxan-induced diabetic rats.

Acknowledgement

The authors are thankful to the Principal, KLE College of Pharmacy, Vidyanagar, and Hubballi, for all facilities to do this study.

Reference

1. Sada NM, Tanko Y, Mabrouk MA. Modulatory role of soya beans supplement on lipid profiles and liver enzymes on alloxan-induced diabetic Wistar rats: Eur J Exp Bio. 2013; 3(2): 62-7
2. Lyra R, Oliveira M, Lins D, Cavalcanti N. Prevention of type 2 diabetes mellitus: Arquivos Brasileiros de Endocrinologia & Metabologia. 2006; 50(2): 239–249.
3. Rajaei Z, Hadjzadeh MAR, Moradi R, Ghorbani A, Saghebi A. Antihyperglycemic and antihyperlipidemic effects of hydroalcoholic extract of Securigera securidaca seeds in streptozotocin-induced diabetic rats: Adv. Biomed. Res. 2015; 4(33): 1-15.
4. Global report on diabetes. World health organization; 2016.
5. Manisha M, Priyanjali D, Jayant L, Saroj G, Thomas PA. Devasagayam. Indian herbs and herbal drugs used for the treatment of diabetes: J. Clin. Biochem. Nutr. 2007; 40(3): 163–73.
6. Ali H, Houghton PJ, Soumyanath A. Alpha-Amylase inhibitory activity of some Malaysian plants used to treat diabetes; with particular reference to Phyllanthus amarus: J. Ethnopharmacol. 2006; 107(3): 449–455.
7. He CN, Wang CL, Guo SX. Study on chemical constituents in herbs of Anoectochilus roxburghii II: Chin J Chin Materia Medica. 2005; 30(10): 761–63.
8. Wright JR, Abraham C, Dickson BC, Yang H, Morrison CM. Streptozotocin dose–response curve in tilapia, a glucose–responsive teleost fish: Gen. Comp. Endocrinol. 1999; 114: 431–40.
9. Suchitra M, Binoy VC. "*Vitex trifolia*: An Ethnobotanical and Pharmacological Review": Asian Journal of Pharmaceutical and Clinical Research. 2018; 11(4): 12-14.
10. Sanjeev K Kumari S, Nayan K P. Diabetes Mellitus and Allopathic Medication Increase the Risk of Cancer Malignancy, but no Side Effect Associated with the Use of Antidiabetic Herbal Medicine. Curre Res Diabetes & Obes J 2020; 13(4): 555868: 47-64.
11. B.Jayaprasad, D.Thamayandhi and P.S.Sharavanan. Traditionally using anti-diabetic medicinal plants in tamil nadu. International Journal of Research in Pharmaceutical and Biosciences 2012: 2(1): 1-8.
12. Sarla Saklani, Abhay Prakash Mishra, Harish Chandra. Comparative Evaluation of Polyphenol Contents and Antioxidant Activities between Ethanol Extracts of *Vitex negundo* and *Vitex trifolia*. Leaves by Different Methods. Plants (Basel). 2017: 6(4): 45.
13. Prasanna Raja.P, Sivakumar. V, Riyazullah. M. S. Antidiabetic Potential of Aqueous and Ethanol Leaf Extracts of *Vitex negundo*. International Journal of Pharmacognosy and Phytochemical Research 2012; 4(2); 38-40.
14. M Ono, H Sawamura, Y Ito, K Mizuki, T Nohara. Diterpenoids from the fruits of *Vitex trifolia*. Phytochemistry, 2000: 55(8): 873-7.
15. A.Nagarajan and P. Brindha. Diterpenes-A Review on Therapeutic uses with special emphasis on Antidiabetic Activity. Journal of Pharmacy Research 2012,5(8),4530-4540.
16. Atsuyoshi Nishina, Masaya Itagaki,1 Daisuke Sato, Hirokazu Kimura, Yasuaki Hirai, Nyunt Phay and Makoto Makishima. The Rosiglitazone-Like Effects of Vitexilactone, a Constituent from *Vitex trifolia* L. in 3T3-L1 Preadipocytes. Molecules. 2017 Nov; 22(11): 2030.
17. Farnsworth NR. Biological and phytochemical screening of plants: J Pharm Sci. 1966; 55(3): 225-76.
18. Fard MH, Naseh G, Lotfi N, Hosseini SM, Hosseini M. "Effects of aqueous extract of turnip leaf (*Brassica rapa*) in alloxan-induced diabetic rats": Avicenna Journal of Phytomedicine. 2015; 5(2): 148-56.
19. Suryawanshi NP, Bhutey AK, Nagdeote AN, Jadhav AA, Manoorkar GS. Study of lipid peroxide and lipid profile in diabetes mellitus: Indian J Clin Biochem. 2006; 21(1): 126–30.
20. Abbate SL, Brunzell JD. Pathophysiology of hyperlipidemia in diabetes Mellitus: J of Cardiovascular Pharmacology. 1990; 16(9): 1–7.
21. Arora M, Koley S, Gupta S, Sandhu JS. A study on lipid profile and body fat in patients with diabetes mellitus: Anthropologist. 2007; 9(4): 295–98.

22. Moller N, Nair KS. Diabetes and protein metabolism: Diabetes. 2008;57(1):3–4.
23. Friel K. The Effects and Interactions of Vitamin D Deficiency, Calcium and Parathyroid Hormone on Physical Concerning the Elderly: A Review. J Geriatr Phys Ther. 2004;27(1):30–34.
24. Bamidele O, Arokoyo DS, Akinnuga AM, Oluwarole AO. Anti-diabetic effect of aqueous extract of Basella alba leaves and metformin in alloxan-induced diabetic albino rats: African journal of Biotechnology. 2014;13(24):2455-58
25. Jeedi NM, Koti BC. Anti-diabetic and antihyperlipidemic activity of Premna latifolia extract in streptozotocin-induced diabetic rats: International Journal of Research in Pharmaceutical Sciences. 2017;8(4):708-15
26. Barham D, Trinder P. An improved colour reagent for the determination of blood glucose by the oxidase system: Analyst. 1972;97(151): 142–5.
27. Tietz NW. Ashwood C editor. Textbook of clinical chemistry. Edn 3, W B Saunders, 1999, pp. 826-835.
28. Fossati P, Prencipe L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide: Clin Chem. 1982; 28(10): 2077–80.
29. McGowan MW, Artiss JD, Strandbergh DR, Zak B. A peroxidase-coupled method for the colorimetric determination of serum triglycerides: Clin Chem. 1983; 29(3): 538–42.
30. Rifai ND, Warnick GR, Dominiczak MH. Handbook of Lipoprotein Testing. Edn 2, AACC Press, Washington, 1997, pp. 99-114.
31. Michael ML. The Measurement of Total Serum Proteins by the Biuret Method: Annals of Clinical and Laboratory Science. 1978: 8(2); 106-10.
32. Morgan BR, Artiss JD, Zak B. Calcium determination in serum with stable alkaline Arsenazo III and triglyceride clearing: Clinical Chemistry. 1993; 39(8): 1608–12.
33. Fogh-Andersen N, McNair P, Møller-Petersen J, Madsbad S. Serum calcium fractions in diabetes mellitus: Clinical chemistry. 1982; 28(10): 2073-76.

HOW TO CITE THIS ARTICLE: Naikwad HM, Jeedi NM. Effect of *Vitex trifolia* on alloxan induced diabetic rat. Int. J. Pharm. Sci. Drug Res. 2021;13(1):9-14.
DOI: 10.25004/IJPSDR.2021.130102