Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/9f4yfk46

EFFECTIVENESS OF ATORVASTATIN & ITS COMBINATION WITH MORINGA OLEIFERA IN DIABETIC HYPERLIPIDEMIC RATS

Sana Imran[1*], Syeda Afroz[2], Tahira Mujtaba[3], Syeda Noor[2], Sumaira Khadim[4], Saroj Fatima[5], Muhammad Sair[6] and Azmat Ara[7]

[1*]Department of Pharmacology, Jinnah Sindh Medical University, Karachi, Pakistan.
[2] Department of Pharmacology, Faculty of Pharmacy, University of Karachi, Karachi, Pakistan.
[3] College of Health & Life Sciences, Aston University, Birmingham UK.
[4] Iqra University, Karachi, Pakistan.
[5] Baqai Institute of Diabetology and Endocrinology, Karachi, Pakistan
[6] Department of Pharmacology, M Islam Medical College, Gujranwala.
[7] Department of Pharmaceutics, University of Karachi, Karachi, Pakistan.

***Corresponding Author:** Sana Imran
*Department of Pharmacology, Jinnah Sindh Medical University, Karachi, Pakistan.
http://orcid.org/0000-0001-8678-7120

ABSTRACT
Diabetes mellitus is a metabolic disease due to lessened secretion of insulin from beta cells of the pancreas resulting in abnormal biotransformation of proteins, fats, and carbohydrates. To assess the advantageous outcome of Atorvastatin and its combination with *Moringa oleifera* in Wistar rats suffering from diabetes mellitus & hyperlipidemia. In a 42-day study, a total of twenty-four Wistar rats were placed in four groups, and in each group, there were six rats. The Control group (Non-Diabetic, Non-Hyperlipidemic) was Group 1, the Control group (Untreated Diabetic & Hyperlipidemic) was Group 2, the Diabetic & Hyperlipidemic group treated with Atorvastatin was Group 3 while the Diabetic & Hyperlipidemic group treated with Atorvastatin & *Moringa oleifera* in combination was Group-4. In regulating hyperglycemia & hyperlipidemia, combination therapy of Atorvastatin & *Moringa oleifera* proves a better option. This sums up the evolvement of add-on therapy in managing diabetes mellitus & hyperlipidemia.

Keywords: Diabetes mellitus, Hyperlipidemia, Moringa oleifera, Atorvastatin

INTRODUCTION
Diabetes mellitus (DM) is a long-standing metabolic condition. It commences with the lysis of pancreatic beta cells with decreased insulin secretion and desensitization of receptors responsible for the action of insulin, resulting in a disturbed normal metabolic process of proteins, fats, and carbohydrates.[1] In 1936, a marked distinction was made between T1DM & T2DM (Type-1 & Type-2 Diabetes mellitus respectively).[2] T2DM is a lifestyle health issue that gives rise to hyperglycemia, development of insulin resistance, and relative deficiency of insulin. [3] It is assumed that T2DM is predominantly due to genetics and modifications in lifestyle. [4] The abundance of any class of plasma lipids portrays Hyperlipidemia (Hyper-LP). Lipid metabolism pathways are affected in DM due to deficiency of insulin production & secretion. [5]

THERAPY FOR TYPE-2 DIABETES MELLITUS (T2DM) & HYPERLIPIDEMIA (Hyper-LP)

As obesity is strongly inherited and is an independent hazard for T2DM [6] therefore, remedy from non-pharmacological methods such as modification in diet & aerobic exercises and natural or chemically synthesized pharmacological drugs such as intake of Insulins, Biguanides & Statins show considerable upturn glycemic control and lipid profile.[7, 8]

Herbal therapy either edible or non-edible, also gains importance in treating various diseases like cough, cold, fever, DM, Hyper-LP, joint pain, asthma, etc.[9] Hence, research on combining pharmacological drugs with herbal therapy is now raised to overcome the drawbacks of pharmacological medications and to improve beneficial effects in profile on blood glucose and lipid samples in experimental studies.[10]

Moringa oleifera's (MO) leaves, flowers, fruits, seeds, and roots have been used as food and in traditional medicine.[11] Recently, MO has shown benefits in numerous diseases including neurological & gastroenterological, diabetes mellitus, hyperlipidemia, cardiovascular, cancer, etc.[12]

Atorvastatin (ATV) belongs to statins. This standard drug constraints rate restricting enzyme HMG-CoA reductase which converts the HMG-CoA to Mevalonic acid and surges the Low-Density Lipoprotein (LPL) receptors. Increased LDL receptors increase the attachment of circulating LDL and decrease its plasma accumulation.[13] This helps to treat Hyper-LP. However, statins produce inert or no response on blood glucose levels if taken in mild to moderate doses with or without T2DM.[14]

METHOD

The study was conducted after ethical approval from ASRB (ASRB No/ 05848 /Pharm) in the Pharmacology Department, at the University of Karachi.

PROCEDURE OF *MORINGA OLEIFERA* LEAF EXTRACT

Fresh, uninfected, and healthy leaves of *Moringa oleifera* were delivered in zip-lock bags to the Pharmaceutical Laboratory at the University of Karachi.

Once at the laboratory, any dust present on the leaves was smoothly washed away using tap water, without damaging them. Subsequently, the cleaned leaves were placed at room temperature in a shade-drying area for two weeks. Afterward, they were pulverized into fine particles using an electric mincer to make a leaf powder. A portion of the pulverized leaf powder was soaked in 80% ethanol in stoppered flasks. The flasks were then placed for 48 hours to boost extraction efficiency. Later, the suspensions are filtered using Whatman no. 1 to remove debris. The filtered extracts are subjected to evaporation using a rotary evaporator until they reach dryness at room temperature, resulting in solid extracts. The solid extracts are dissolved in 30% dimethyl sulfoxide (DMSO) to obtain a stock solution with a concentration of 100 mg/mL

GROUPING & DOSING OF WISTAR RATS

A total of twenty-four male healthy Wistar rats of 150-200 grams in body weight were bought from the animal house of Dow University of Health Sciences (DUHS). They were given a normal diet and water for one week for familiarization before research work. Afterward, Wistar rats were randomly & equally distributed into four groups for experimental studies. The Control / Non-Diabetic & Non-Hyperlipidemic group was labeled as Group-1, Control / Diabetic & Hyperlipidemic untreated group was labeled as Group-2, Diabetic & Hyperlipidemic - Atorvastatin (ATV) treated group was labeled as Group-3 and Diabetic & Hyperlipidemic – Atorvastatin (ATV) & *Moringa oleifera* (MO) in combination-treated group was labeled as Group-4.

Dexamethasone (DEXA) 10mg/cc was injected subcutaneously for the induction of Hyper-LP in Group-2, Group-3 & Group-4 of Wistar rats for eight days.[15] A single dose of freshly prepared Streptozotocin (STZ) of 60 mg/Kg body weight in citrate buffer (pH 4.5) was also inoculated intraperitoneally for the induction of T2DM in the same groups of Wistar rats.[16] Blood samples

were assembled after eight days of Inj. DEXA therapy, equivalent to seventy-two hours of Inj. STZ from the tip of the rat's tail for confirmation of raised fasting blood glucose (FBS) level & cardiac puncture for determination of raised HbA1c & Hyper-LP. Rats with raised lipid profile & blood sugar (FBS & HbA1c) values were used for research work.

After confirmation of induction of T2DM & Hyper-LP in Groups-2, 3 & 4, the stock solution of ATV 20 mg/Kg rat's body weight making a dosage of 0.06 mg was given in Group-3, while that of MO ethanolic leaf extract 200 mg/Kg rat's body weight making a dosage of 0.6 mg was given along with ATV in Group-4.

The dosage of drugs was selected according to the initial weight of research rats that is before induction of T2DM & Hyper-LP. The selected dosage was given per oral through a standard syringe once daily in the morning for 42 days.[17]

Blood sugar levels (FBS & HbA1c) & Lipid profile [Total Cholesterol (TC), Triglyceride (TG), High-Density Lipoprotein (HDL-C) & Low-Density Lipoprotein (LDL-C)] were biochemical parameters. They were measured at Day 0 (Post-induction time of T2DM & Hyper-LP, before drug therapy) & Day 42 (Post-induction time of T2DM & Hyper-LP, following drug therapy) for analysis & comparison of FBS, HbA1c & Hyper-LP among different groups in animal experimental research work.

In all four groups, two animals were slaughtered on Day 0 while the rest of the animals were slaughtered on Day 42 for determination & comparison of biochemical values.

The main objective of the research work was to judge the gainful consequence of Atorvastatin 20mg with or without *Moringa oleifera* in the management of induced hyperglycemia & hyperlipidemia in Wistar rats.

The data feeding and analysis after research was done on SPSS 20 (Statistical packages of social science). In all statistical analysis the consideration of p-value <0.05 was significant.

RESULTS

Research work on Group – 3 showed that ATV was not effective in the treatment of hyperglycemia (FBS & HbA1c) when given from Day 0 to Day 42. Instead, ATV further enhanced blood sugar levels as shown in Table 1 and Figure 1. On the other hand, Group – 4 revealed that the combination of ATV & MO produced effectual consequences in the management of T2DM from Day 0 to Day 42 in hyperglycemia & hyperlipidemia-induced Wistar rats as depicted in Table 1 and Figure 2.

Table 1 COMPARISON OF PERCENTAGE CHANGES IN MEAN FBS & HbA1c IN DIFFERENT EXPERIMENTAL GROUPS FROM DAY 0 -DAY 42

PARAMETER	PERCENTAGE CHANGE	p-VALUE	PARAMETER	PERCENTAGE CHANGE	p-VALUE
FBS (mg/dL) Group - 1	1.16%	0.5869	**HbA1c (%)** Group - 1	1.11%	0.3125
Group – 2	59%	0.0001*	Group – 2	76.69%	0.0004*
Group – 3	32%	0.0001*	Group – 3	62.38%	0.0015*
Group – 4	- 36.65%	0.0001*	Group – 4	30.9%	0.0001*

Values are expressed in Mean ± S.D

S.D = Standard Deviation

* = Significant

GROUP 1: Control Group (Non-Diabetic, Non-Hyper-LP)

GROUP 2: Control Group (Untreated Diabetic, Hyper-LP)

GROUP 3: Atorvastatin 20mg treated Group

GROUP 4: Atorvastatin 20mg & MO treated Group

Figure 1

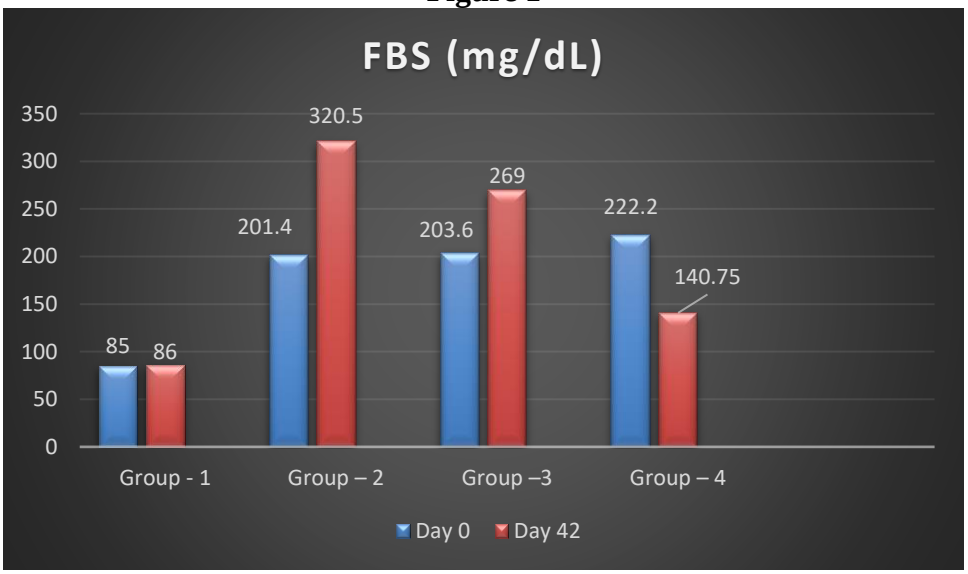
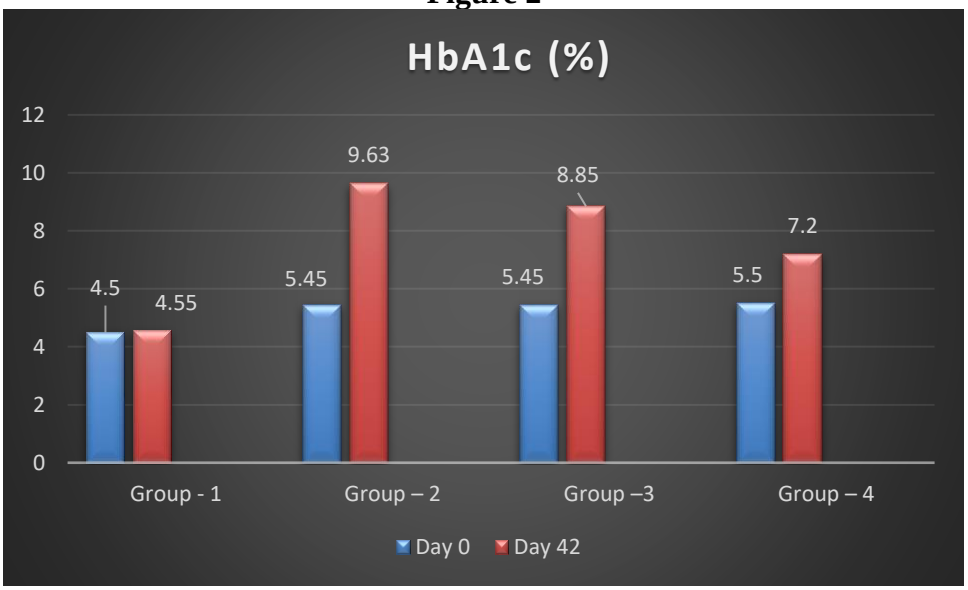


Figure 2



When comparing Group – 4 with Group – 3, it was exhibited both groups were proficient in the remedy of Hyper-LP (TC, TG, HDL-C & LDL-C) when given from Day 0 to Day 42. However, Group – 3 displayed better outcomes in raising HDL levels while Group – 4 revealed improved results in lowering TC & TG levels. In the case of LDL-C level, both Group – 3 & Group – 4 showed more or less the same results in hyperglycemia & hyperlipidemia-induced Wistar rats as proven in Table 2 and Figures 3, 4. 5 & 6.

Table 2 COMPARISON OF PERCENTAGE CHANGES IN MEAN LIPID PROFILE IN DIFFERENT EXPERIMENTAL GROUPS FROM DAY 0 -DAY 42

PARAMETER	PERCENTAGE CHANGE	p-VALUE
TOTAL CHOLESTEROL (mg/dL) Group - 1	11.51%	0.0815
Group - 2	25.65%	0.0004*
Group - 3	- 12.7%	0.0003*

Group - 4	- 25.54%	0.0001*
TRIGLYCERIDE (mg/dL) Group - 1	1.4%	0.8499
Group - 2	26.18%	0.0023*
Group - 3	- 25.17%	0.0002*
Group - 4	- 31.61%	0.0002*
HDL (mg/dL) Group - 1	- 1.6%	0.8156
Group - 2	- 30.64%	0.0017*
Group - 3	31.63%	0.0002*
Group - 4	29.12%	0.0004*
LDL (mg/dL) Group - 1	19.06%	0.0028*
Group - 2	40.12%	0.0017*
Group - 3	- 17.21%	0.0003*
Group - 4	- 17.32%	0.0005*

Values are expressed in Mean ± S.D
S.D = Standard Deviation
* = Significant
GROUP 1: Control Group (Non-Diabetic, Non-Hyper-LP)
GROUP 2: Control Group (Untreated Diabetic, Hyper-LP)
GROUP 3: Atorvastatin 20mg treated Group
GROUP 4: Atorvastatin 20mg & MO treated Group

Figure 3

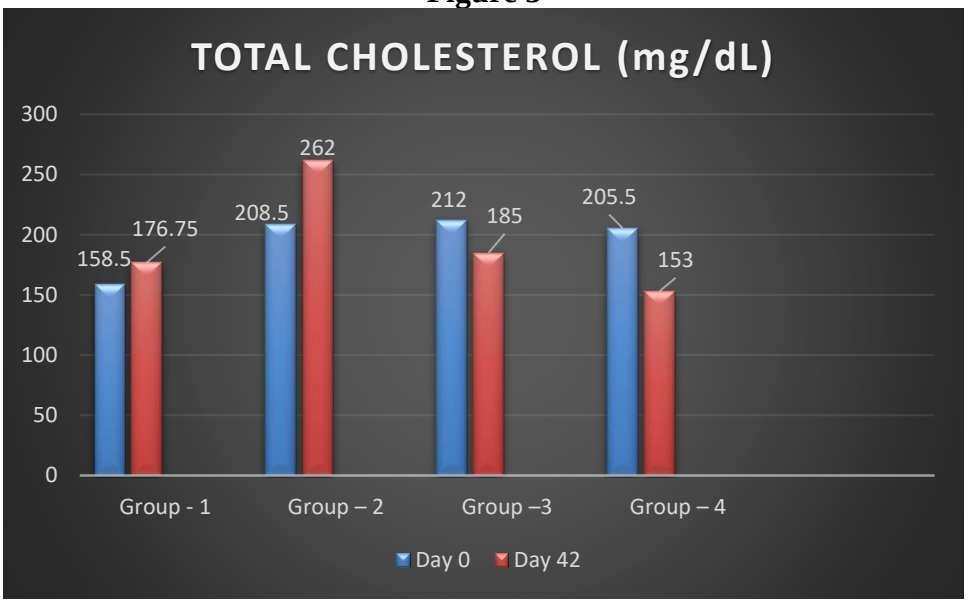


Figure 4

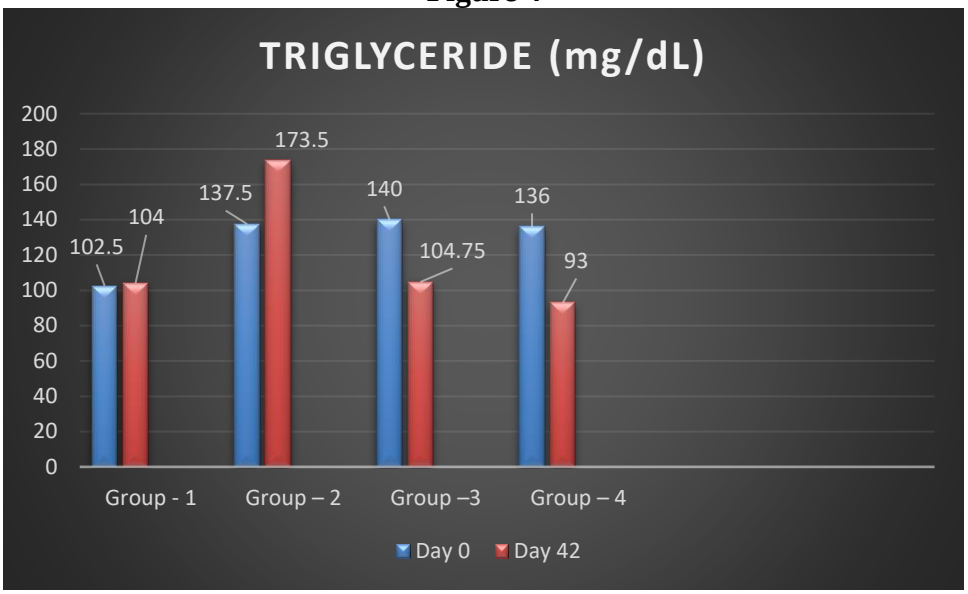


Figure 5

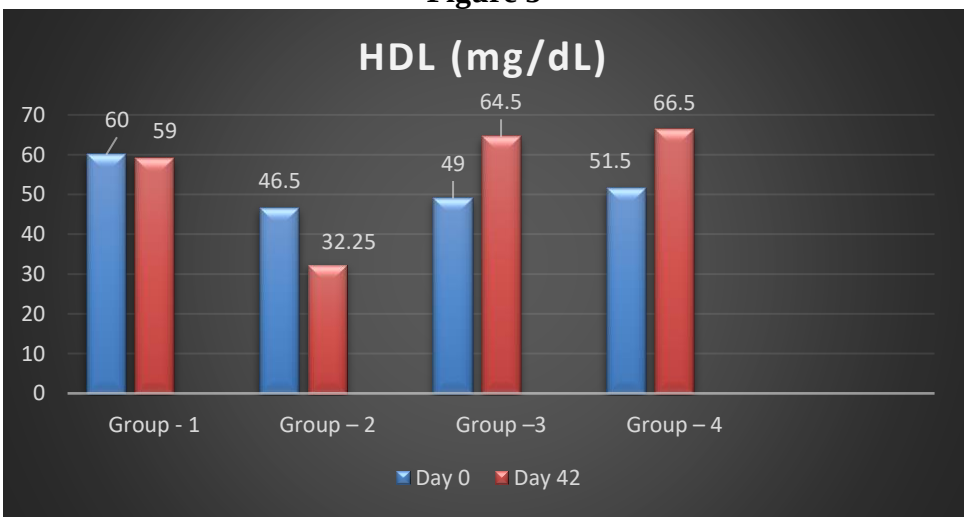
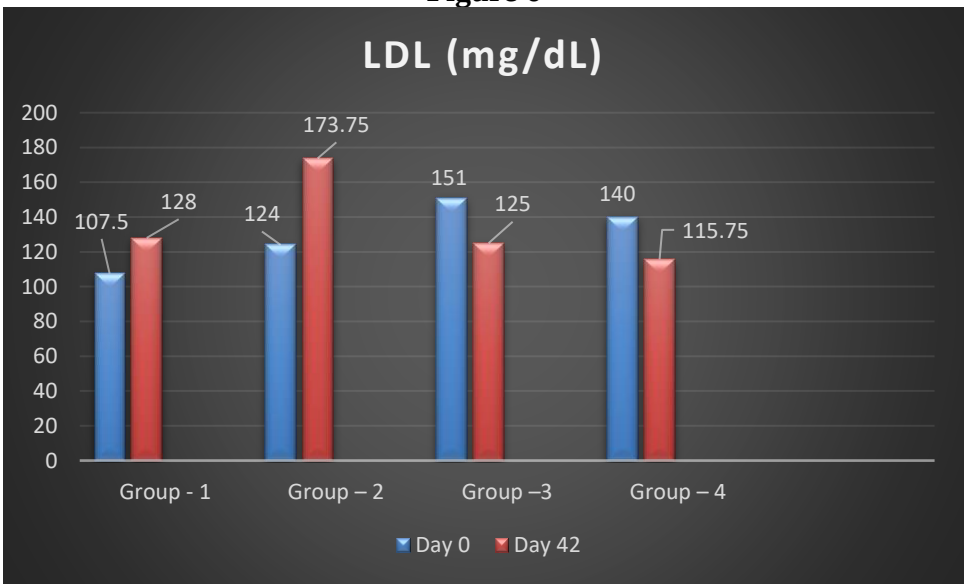


Figure 6



DISCUSSION

The objective of this 42-day research work was to compare the therapeutic effects of ATV alone & in combination with MO in Wistar rats diseased from T2DM & Hyper-LP.

In this research project conducted between Day 0 (start of therapy) and Day 42 (end of therapy), the trivial effects in controlling T2DM (FBS & HbA1c) in induced hyperglycemia & hyperlipidemia Wistar rats were seen in ATV 20mg treated Group – 3 & ATV 20mg & MO in combination-treated Group – 4.

In research work performed by Al-Bayyari et al (2017),[18] the increased FBS level was observed in patients receiving ATV 20mg that is in concurrence with our ATV 20mg treated Group – 3. Similarly, in the 2-month research study of Koh et al (2010),[19] the increment of HbA1c was seen in patients taking ATV 20mg which is in correspondence to our ATV 20mg treated Group – 3.

The research of Oyedepo et al (2013), [20] depicted the curative effect of MO in regulating T2DM. It suggests that the combination of ATV & MO would be beneficial in the therapy of T2DM which is in accord with our ATV 20 mg & MO in combination-treated Group – 4.

Conversely in this research project, both ATV 20mg treated Group – 3 & ATV 20mg & MO in combination with treated Group – 4 produced proficient outcomes in controlling Hyper-LP when given from Day 0 to Day 42.

In the study done by Liu P-Y et al (2013), [21] slight decrements in HDL-C levels were noted while in the case of TC, TG, & LDL-C levels, notable decrement was observed respectively, among patients taking ATV 10mg for 12 weeks which is in accordance to our ATV 20mg treated Group – 3. The 12-week study of Kunj et al (2020) [22] conducted among patients taking ATV 10 mg & MO in combination, showed a reduction of TC & LDL levels respectively while an upsurge of HDL level was also observed that is in compliance with our ATV 20 mg & MO in combination-treated Group – 4.

CONCLUSION

From this research, it is depicted that combination therapy of ATV & MO is a far better choice in diabetic hyperlipidemic Wistar rats, bringing up new hope in the management of T2DM & Hyper-LP in human beings.

CONFLICT OF INTEREST

None

REFERENCES

1. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes care. 2010; 33: 62-69.
2. Diabetes mellitus history- from ancient to modern times. Available at http:// science.jrank.org/pages/2044/Diabetes-Mellitus.html. (accessed on 22nd July,2011).
3. Maitra A, Abbas AK. Endocrine system. In: Kumar V, Fausto N, Abbas AK (eds). Robbins and Cotran Pathologic basis of disease (7th ed) 2005. Philadelphia, Saunders; 1156-226.
4. Ripsin CM, Kang H, Urban RJ. Management of blood glucose in type 2 diabetes mellitus. Am Fam Physician Jan 2009; 79:29-36.
5. Maryam Munawar, Shoaib Asif, Haroon Habib, et al. Outlook on anti-hyperlipidemic outcome of *Moringa oleifera* in relationship with Statins. International Journal of Biology, Pharmacy & Applied Sciences (IJBPAS) June 2019; 8:1165-76.
6. Walley AJ, Blakemore AI, Froguel P. Genetics of obesity and the prediction of risk for health. Hum Mol Genet Oct 2006; 15:124-30.
7. American Diabetes Association. 8. Pharmacologic approaches to glycemic treatment. Diabetes Care. 2017; 40:64-74.
8. Thomas S. Medications that Lower Cholesterol. Journal of Lipid Research. 2003; 33: 79-82.

9. Keskin C, Özen HC, Toker Z, et al. Determination of in vitro antioxidant and antimicrobial properties of shoot and root extracts of Astragalus diphtherites FENZL var. diphtherites and Astragalus gymnalopecias RECH. FIL. obtained by different solvents. Kahramanmaras Sutcu Imam University Journal of Natural Sciences. 2018; 21:157-66.
10. Mohamed-Hesham Daba, Ahlam Elmasry, A. El-karef. Effect of *Moringa oleifera* with and without metformin on an experimental model of metabolic syndrome in rats. Int'l J. of Adv. Res. 2015;3(10):1624-32.
11. Popoola, J.O., Obembe, O.O. Local knowledge, use pattern and geographical distribution of *Moringa oleifera* Lam. (Moringaceae) in Nigeria. J. Ethnopharmacol. 2013; 150: 682–91.
12. Anwar, F., Latif, S., Ashraf, M., et al. Moringa oleifera: A food plant with multiple medicinal uses. Phyther. Res. 2007; 21:17–25.
13. Sage, AP, Lu J, Atti E, et al. Hyperlipidemia induces resistance to PTH bone anabolism in mice via oxidized lipids. J. Bone Miner Res. 2011; 26: 1197-206.
14. Freeman DJ, Norrie J, Sattar N et al. Pravastatin and the development of diabetes mellitus: evidence for a protective treatment effect in the West of Scotland Coronary Prevention Study. *Circulation* 2001; 103:357-362.
15. N. Sriram, Rajaneeekar. D, Kameshwaran. S et al. Antihyperlipidemic activity of flower extract of Tecoma stans against dexamethasone-induced hyperlipidemia in rats. IJAMSCR. 2020; 8(3): ISSN: 2347-6567
16. Nagmoti DM, Kothavade PS, Bulani VD et al. Antidiabetic and antihyperlipidemic activity of Pithecellobium dulce (Roxb.) Benth seeds extract in streptozotocin-induced diabetic rats. Eur J Integr Med 2015; 7:263e73.
17. Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain. 1983 Jun; 6(2):109-110. doi: 10.1016/0304-3959(83)90201-4. PMID: 6877845.
18. Al-Bayyari N, Saadeh N, Hailat R, Al-Zeidaneen S. Assessment of Atorvastatin effect on body weight and blood glucose levels among Diabetic and non-Diabetic patients. Rom J Diabetes Nutr Metab Dis. 2017; 24(3):255-262. doi: 10.1515/rjdnmd-2017-0031
19. Koh KK, Quon MJ, Han SH, Lee Y, Kim SJ, Shin EK. Atorvastatin causes insulin reistance and increases ambient glycemia in hypercholesterolemic patients. J Am Coll Cardiol. 2010 Mar 23;55(12):1209-1216. doi: 10.1016/j.jacc.2009.10.053. PMID: 20298928; PMCID: PMC3016087
20. Oyedepo T.A, Babarinde S.O, Ajayeoba T.A. Evaluation of Anti-hyperlipidemic Effect of queous Leaves Extract of *Moringa oleifera* in Alloxan Induced Diabetic Rats. Int'l J of Biochem Research & Review 2013;3(3):162-170.
21. Liu P-Y, Lin L-Y, Lin H-J, Hsia C-H, Hung Y-R, et al. Pitavastatin and Atorvastatin Double-Blind Randomized ComPArative Study among HiGh-Risk Patients, Including ThOse with Type 2 Diabetes Mellitus, in Taiwan (PAPAGO-T Study). PLoS ONE. 2013; 8(10): e76298. doi:10.1371/journal.pone. 0076298. ISSN: 1932-6203
22. Kunj M., Keshri USP., Kumar C., Rana R., Kapoor S., Kumar A. Therapeutic Benefits of Mthanolic Extract of *Moringa oleifera* Leaf Extract in Dyslipidemia: Observational Comparative Follow up Study. Sch Acad J Biosci, Dec, 2020; 8(12): 417-422 ISSN 2347-9515 (Print) | ISSN 2321-6883 (Online) https://saspublishers.com