IJPSR (2012), Vol. 3, Issue 11 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 20 July, 2012; received in revised form 14 September, 2012; accepted 23 October, 2012

ANTI-HYPERGLYCEMIC ACTIVITY OF SIMVASTATIN ALONE (THERAPEUTIC DOSE) AND COMBINATION OF SIMVASTATIN AND GLIPIZIDE (SUB THERAPEUTIC DOSES) ON ALLOXAN INDUCED HYPERGLYCEMIA IN ALBINO RATS

V.S. Harish Kumar*, N.R. Sindhu, Rajashri S. Patil and Umakant Patil

S. S. Institute of Medical Sciences & Research Centre, Davangere, Karnataka, India

Keywords:
Simvastatin,
Anti-hyperglycemic,
Glipizide,
Alloxan

Correspondence to Author:

V.S. Harish Kumar

Assistant Professor, Department of Pharmacology, S.S.I.M.S & R.C., NH-4, Bypass road, Davangere-577005, Karnataka, India

E-mail: harishkvspharma@gmail.com

QUICK RESPONSE CODE

IJPSR:
ICV (2011)- 5.07

Website:
www.ijpsr.com

ABSTRACT

The present study was designed to evaluate anti-hyperglycemic activity of simvastatin alone and the combination of sub therapeutic doses of simvastatin and glipizide. Hyperglycemia was induced experimentally in albino rats by subcutaneous injection of alloxan in a dose of 175 mg/kg body weight. After 72 hours of alloxan treatment, rats showing hyperglycemia (blood glucose level of 400 mg/dl and above) were included in the study. They were divided into four groups of 6 animals each (n=24). Oral administration of normal saline 0.5 ml, glipizide 2.5 mg/kg body weight, simvastatin 10 mg/kg body weight and sub therapeutic doses of both test (simvastatin 5 mg/kg body weight) and standard (glipizide 1.25 mg/kg body weight) drugs, was done respectively into each of the four groups for 30 consecutive days in order to assess the effect in terms of reduction in blood glucose level. Blood glucose was estimated on 0^{th}, 10^{th}, 20^{th}, and 30^{th} days of study in fixed time intervals. In the test group, there was a gradual fall in the blood glucose level which reached up to 308.3 mg/dl by 30^{th} day of study ($P < 0.001$). In case of combination of sub therapeutic doses of simvastatin and glipizide, the fall in blood glucose level was gradual and sustained and it reached up to 201.5 mg/dl by the 30^{th} day ($P < 0.001$). These observations are comparable with the results obtained in case of glipizide treated rats, the standard group. Simvastatin appreciably lowered the blood glucose level, but the combination of sub therapeutic doses of simvastatin and glipizide, by virtue of their possible synergistic effect produced further reduction in the blood glucose level. This study provides evidence in support of a potential anti-hyperglycemic effect of simvastatin and its combination with glipizide. Thus the combined treatment of simvastatin and glipzide may have added benefit for the diabetic patients associated with hyperlipidemia.

INTRODUCTION: Diabetes mellitus (DM) is the world's largest endocrinal disorder and a major threat to the global public health. It involves metabolic abnormalities of carbohydrates, fats and proteins. This metabolic disorder is characterized by hyperglycemia, associated with accelerated atherosclerosis leading to increased risk and incidence of myocardial infarction (MI), stroke and peripheral arterial disease. Diabetes is also the leading cause of adult blindness and end-stage renal disease.

If glycemic controls are achieved satisfactorily by therapeutic and non-therapeutic approaches, it is possible to reduce the progression of atherosclerosis, stabilize rupture-prone plaques and thus prevent arterial thromboembolism and eventually prevent cardiac death due to MI. The concomitant incidence of retinopathy and nephropathy can also be minimized. Overall therapeutic approaches to manage DM include usage of anti-diabetic drugs and anti-hyperlipidaemic drugs.

Simvastatin, an anti-hyperlipidemic agent belongs to statins family, a class of drugs that competitively inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. This enzyme catalyzes the conversion of HMG-CoA to mevalonate, the rate-limiting step of the cholesterol synthesis pathway [1]. Recently, HMG-CoA reductase inhibitors have been reported to exert various other effects in addition to their lipid-lowering ability. Simvastatin attenuates leukocyte-endothelial cell interactions and subsequent blood-retinal barrier breakdown via suppression of vascular endothelial growth factor-induced intercellular adhesion molecule-1 (ICAM-1) expression in the diabetic retina. It may thus be useful in the prevention of diabetic retinopathy [2].

Simvastatin is the mainstay in the management of hyperlipidemia. Clinical trials indicated that diabetics profit from this agent [3] as it is not uncommon to see both conditions co-existing.

There have been very few studies on simvastatin as an anti-hyperglycemic agent and no study has investigated on its combination with glipizide for anti-hyperglycemic effect. Hence the present study was designed to evaluate the anti-hyperglycemic effect of simvastatin alone as well as its combination effect with glipizide in sub therapeutic doses, and to compare its anti-hyperglycemic effect with therapeutic dose of glipizide, a standard drug which is one of the sulfonylureas. This emphasis may widen the therapeutic horizon for the said agent.

MATERIALS AND METHODS:

A. **Drugs and chemicals**: Simvastatin, Glipizide, Alloxan, Dextrose, Ether.

B. Other requirements: Heparinized micro capillary tubes and Glucometer (ACCU-CHEK ACTIVE) with strips, Manufactured by- Roche Diagnostics GmbH, D-68298 Mannheim, Germany, Batch No-91000247.

C. **Ethical clearance**: The study was conducted in accordance with the National Institute of Health guidelines for the care and use of animals in research, and the protocol was approved by the Institutional Animal Ethical Committee, S. S. Institute of Medical Sciences and Research Centre, Davangere, Karnataka.

D. **Maintenance of rats**: Healthy Albino rats (150-200gm), 12 weeks old of either sex, from the central animal house of S.S.I.M.S & R.C, Davangere, were used for the study. They were maintained under controlled conditions of temp 23±2°C in 12hrs of light and 12 hrs of dark cycles per day. The animals were housed individually in polypropylene cages containing sterile paddy husk as bedding and were fed on normal diet & water *ad libitium*.

E. **Experimental Procedure**:

a. **Induction of Diabetes**- It is done by subcutaneous injection of alloxan in a dose of 175 mg/kg body weight [4, 5]. Rats were treated with 10% dextrose orally to combat the early phase of hypoglycaemia [6]. After 72 hours of alloxan treatment, the blood sugar was estimated and those with blood glucose level of 400mg/dl and above were included in this study.

Groups- The rats were divided into four groups of six animals each and drugs were administered every day for 30 consecutive days.

Group 1 (Control): received normal saline 0.5 ml by oral route daily for 30 days.

Group 2 (Standard): received glipizide 2.5mg/kg body weight by oral route daily for 30 days [7].

Group 3 (Test): received simvastatin10mg/kg body weight by oral route daily for 30 days [8].

Group 4 (Sub therapeutic): received the sub therapeutic doses of both test (simvastatin 5 mg/kg

body weight) and standard (glipizide 1.25 mg/kg body weight) drugs simultaneously by oral route daily for 30 days to assess their combined effect in terms of reduction in blood glucose level.

b. Collection of blood sample and estimation of blood glucose level- Blood samples were collected from the retro-orbital plexus of each rat after induction of mild anesthesia with ether, [9] at the intervals of 0^{th} min (before drug administration), 30^{th} min, 1^{st} hr, 2^{nd} hr, 3^{rd} hr, 6^{th} hr, 12^{th} hr and 24^{th} hr (after the administration of drugs) on 0^{th} day, 10^{th} day, 20^{th} day, 30^{th} day and blood glucose levels were estimated by using glucometer and data were recorded for statistical analysis, which was done using repeated measures ANOVA test. The effect of simvastatin alone and the combination of it with glipizide was compared with the anti-hyperglycemic activity of glipizide, the standard drug.

RESULTS: The rats blood glucose concentration of 400 mg/dl and above, obtained after 72 hrs of alloxan treatment were considered for the present study**.**

Table 1 & chart 1: In the control group of rats receiving normal saline over a period of 30 days, there was no much variation in the blood glucose level. Initially on 0^{th} day, the blood glucose level was 432.5 mg/dl and at the end of 30^{th} day it was 451.2 mg/dl. Though there was a marginal increase in blood glucose compared to the initial level, it was not statistically significant.

Table 2 & chart 2: In the second group treated with glipizide 2.5mg/kg body weight, hyperglycemic rats showed significant fall in blood glucose level from 402.7 mg/dl on 0^{th} day to 166.8 mg/dl on 30^{th} day. This group is considered as the standard.

Table 3 & chart 3: In the test group of simvastatin (10mg/kg body weight) treated rats, there was gradual reduction in the blood glucose level up to 6^{th} hr observation on the respective days of blood glucose estimation. There is a marked fall in the blood glucose level from 404.5 mg/dl on 0^{th} day to 308.3 mg/dl on 30^{th} day, which is statistically highly significant ($P < 0.001$). However the reduction in blood glucose level in this group is not as significant as that observed in the standard group (table 2 & chart 2).

Table 4 & chart 4: In the fourth group of rats receiving the combination of sub therapeutic doses of simvastatin 5 mg/kg body weight and glipizide 1.25 mg/kg body weight over a period of 30 days, there was a gradual and sustained reduction in blood glucose level from 400.7 mg/dl on 0^{th} day to 201.5 mg/dl on 30^{th} day, which is statistically highly significant ($P < 0.001$). Blood glucose levels in this group showed a fall comparable to standard values obtained by treatment with glipizide (table 2 & chart 2).

TABLE 1: CONTROL GROUP - EFFECT OF NORMAL SALINE (0.5 ml) ON BLOOD GLUCOSE (mg/dl) LEVEL OF ALLOXAN INDUCED HYPERGLYCEMIC RATS.

Period	0^{th}min	30^{th} min	1^{st} hr	2^{nd} hr	3^{rd} hr	6^{th} hr	12^{th} hr	24^{th} hr	P*Value, sig
0^{th} Day	432.5±33.4	435.2± 33.7	438.5±33.0	436.0±35.5	437.8±33.5	439.7±31.1	439.2±33.8	439.0±34.0	0.12 NS
10^{th}Day	443.3±31.6	443.5±32.8	442.7±27.4	443.2±33.3	441.3±31.4	442.2±26.9	442.8±28.9	442.7±37.2	1.0 NS
20^{th}Day	442.3±29.2	443.7±30.7	438.3±30.4	443.3±33.2	442.2±31.9	439.3±28.8	443.0±28.1	448.0±32.0	0.12 NS
30^{th}Day	442.5±25.0	444.3±25.9	441.5±25.6	443.8±33.8	442.7±25.2	441.7±19.7	445.5±20.0	451.2±25.1	0.31 NS

Values are means ± SD, * Repeated measures ANOVA test, NS= Non significant.

TABLE 2: STANDARD GROUP - EFFECT OF GLIPIZIDE (2.5 mg/kg body weight) ON BLOOD GLUCOSE (mg/dl) LEVEL OF ALLOXAN INDUCED HYPERGLYCEMIC RATS

Period	0^{th} min	30^{th} min	1^{st} hr	2^{nd} hr	3^{rd} hr	6^{th} hr	12^{th} hr	24^{th} hr	P* Value, sig
0^{th} Day	402.7±10.3	396.8±11.0	388.8±12.1	358.8±10.9	341.5±5.2	328.5±8.5	317.7±8.9	306.3±9.5	<0.001 HS
10^{th}Day	294.2±8.3	293.7±6.2	288.8±8.0	285.2±7.6	282.2±6.4	279.8±7.8	278.2±10.5	272.3±10.9	<0.001 HS
20^{th}Day	265.5±9.2	264.8±8.6	261.5±7.1	253.5±6.4	249.5±6.0	247.2±6.4	243.0±6.4	236.0±9.3	<0.001 HS
30^{th}Day	226.8±4.9	224.7±4.8	216.2±4.1	206.0±4.5	198.3±3.4	190.5±4.1	177.3±4.5	166.8±5.8	<0.001 HS

Values are means ± SD, * Repeated measures ANOVA test, HS= Highly significant.

TABLE 3: TEST GROUP - EFFECT OF SIMVASTATIN (10 mg/kg body weight) ON BLOOD GLUCOSE (mg/dl) LEVEL OF ALLOXAN INDUCED HYPERGLYCEMIC RATS

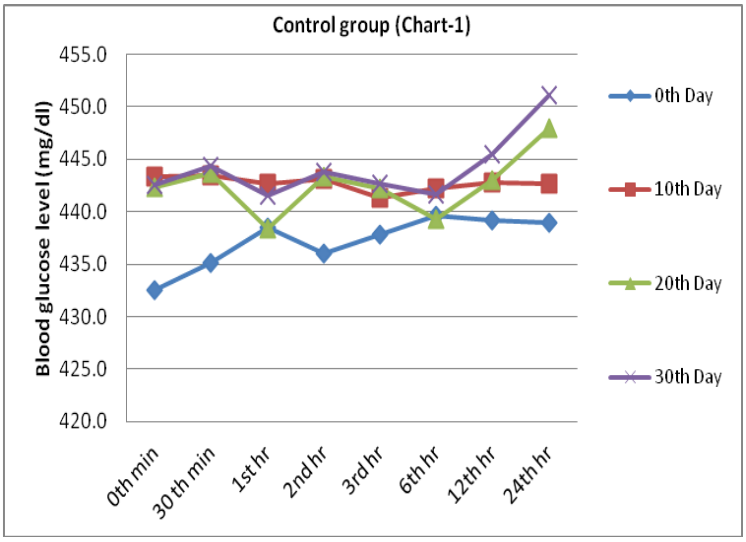
Period	0th min	30 th min	1st hr	2nd hr	3rd hr	6th hr	12th hr	24th hr	P* Value, sig
0thDay	404.5±18.9	403.8±22.1	345.3±39.6	307.3±56.8	303.3±59.0	301.8±61.0	312.5±53.2	365.7±71.8	<0.001 HS
10thDay	361.2±73.3	358.7±73.5	329.8±82.8	299.8±72.2	284.8±70.5	264.8±74.7	302.5±63.0	347.2±66.4	<0.001 HS
20thDay	333.8±61.7	332.8±61.5	320.5±65.5	284.3±73.9	270.5±75.6	256.8±75.9	275.8±76.1	330.5±81.6	<0.001 HS
30thDay	328.8±72.5	327.5±72.1	302.3±68.0	277.5±70.7	263.5±69.3	252.7±72.0	270.5±79.0	308.3±79.9	<0.001 HS

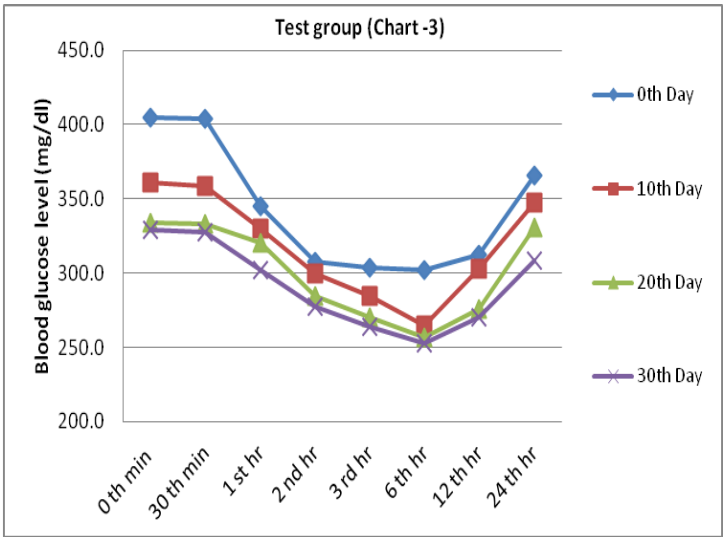
Values are means ± SD, * Repeated measures ANOVA test, HS= Highly significant.

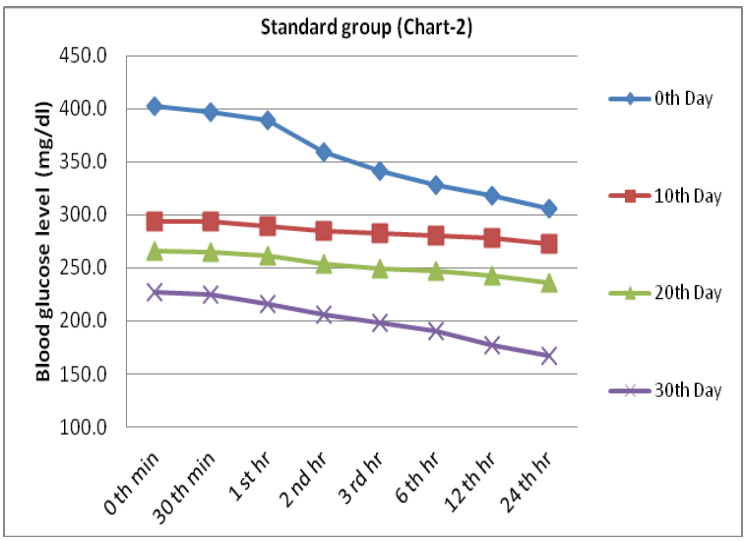
TABLE 4: SUB THERAPEUTIC GROUP - COMBINATION EFFECT OF SIMVASTATIN (5mg/kg body weight) & GLIPIZIDE (1.25 mg/kg body weight) ON BLOOD GLUCOSE (mg/dl) LEVEL OF ALLOXAN INDUCED HYPERGLYCEMIC RATS

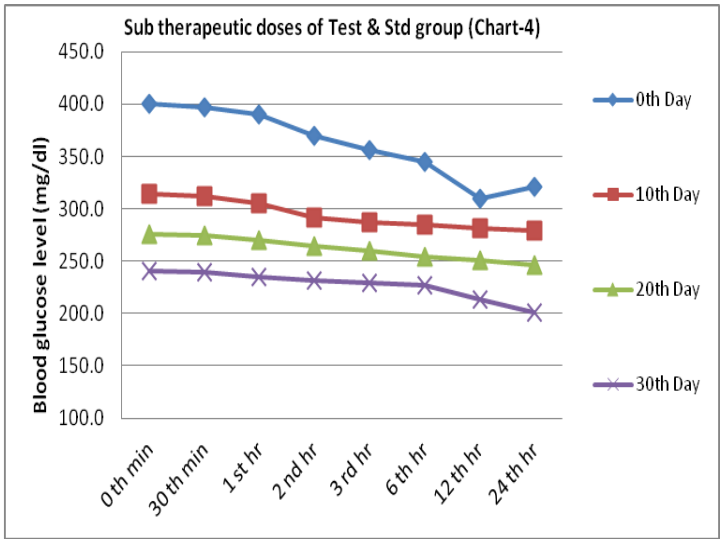
Period	0th hr	30 th min	1st hr	2nd hr	3rd hr	6th hr	12th hr	24th hr	P* Value, sig
0thDay	400.7±19.4	397.2±19.6	389.7±17.9	369.7±21.1	356.5±16.6	345.0±10.5	310.0±10.1	321.3±8.7	<0.001 HS
10thDay	314.7±6.4	312.2±6.0	305.0±6.0	291.5±8.7	287.5±5.2	284.5±4.6	281.3±10.3	279.7±10.7	<0.001 HS
20thDay	275.8±5.4	274.5±6.5	269.8±4.7	264.7±4.2	260.0±3.6	254.2±6.9	250.3±5.7	246.2±5.3	<0.001 HS
30thDay	240.8±3.8	239.7±3.7	235.5±3.5	231.8±3.3	229.5±5.9	227.2±8.4	213.2±9.2	201.5±5.5	<0.001 HS

Values are means ± SD, * Repeated measures ANOVA test, HS= Highly significant.









The combination treatment with sub therapeutic doses of simvastatin and glipizide decreased the blood glucose concentration more than simvastatin treatment alone which is statistically highly significant.

DISCUSSION: As already eluded, diabetes mellitus and hyperlipidemia often coexist and are among the high risk factors for cerebrovascular and cardiovascular morbidity and mortality. HMG-Co A reductase inhibitors have been shown to decrease the risk of cerebrovascular and cardiovascular events [10]. Statins are presently the main stay in the treatment of hyperlipidemia, which on regular use have shown to reduce the rate of morbidity and mortality in the above said disorders.

In the present study, the results obtained by therapeutic dose of simvastatin alone and the combination of it with glipizide in sub therapeutic doses are quite appreciable. With reference to the standard drug, the effect of sub therapeutic dose combination of drugs has given nearly comparable results. Simvastatin reduced the blood glucose level appreciably until 6th hour after which a slight rise in blood glucose, lacking statistical significance was observed. However in case of combination, not even a slight rise in blood glucose level was observed on further readings after the 0th day, indicating persistent effect on the lowering of blood glucose on regular use.

The results definitely indicate additive action of simvastatin on the pharmacodynamic response of glipizide even in doses below their normal therapeutic range. Simvastatin improves the prognosis of diabetic patients with coronary heart disease [11]. It exerts cardio protective effects and improves Insulin resistance, [12] which may be one of the contributing factors. Other studies have also shown multiple benefits of simvastatin like anti-inflammatory action, [13] suppression of oxidative stress [14] and its antioxidant effect [15] that may have role to play.

CONCLUSION: Simvastatin reduces the blood glucose level, whereas the sub therapeutic doses of simvastatin and glipizide in combination will be more effective in lowering the blood glucose concentration, cost beneficial and possibly safer than the individual drug alone as the occurrence of adverse effects will decline with reduction in individual drug dose.

The present observation requires confirmation with further extensive studies. If it is confirmed, it may further rationalize and widen therapeutic utility of simvastatin. Simvastatin is one of the important drugs used for hyperlipidemia. By this study we can emphasize upon the usage of simvastatin and its combination with glipizide, in sub therapeutic doses for the patients of diabetes associated with hyperlipidemia.

ACKNOWLEDGEMENT: We would like to thank Micro Labs Ltd for providing pure drug samples.

CONFLICT OF INTEREST: None declared.

REFERENCES:

1. Tobias Rydgren, Outi Vaarala and Stellan Sandler: Simvastatin Protects against Multiple Low-Dose Streptozotocin-Induced Type 1 Diabetes in CD-1 Mice and Recurrence of Disease in Nonobese diabetic Mice. The Journal of Pharmacology and Experimental Therapeutics 2007; 323: 180-185.
2. Shinsuke Miyahara, Junichi Kiryu, Kenji Yamashiro, Kazuaki Miyamoto, Fumitaka Hirose, Hiroshi Tamura, Hideto Katsuta, Kazuaki Nishijima, Akitaka Tsujikawa and Yoshihito Honda: Simvastatin Inhibits Leukocyte Accumulation and Vascular Permeability in the Retinas of Rats with Streptozotocin-Induced Diabetes. The American Journal of Pathology 2004; 164:5:1697-1706.
3. Brunton LL, Lazo JS and Parker KL, Eds.,: Goodman and Gilman's The Pharmacological Basis of Therapeutics. Mc Graw Hill Publications, 11th edition, 2006:946.
4. H. Gerhard Vogel, Wolfgang H.Vogel, Bernward A. Scholkens, Jurgen Sandow Gunter Muller & Wolfgang F. Vogel: Drug Discovery and Evaluation Pharmacological Assays. Springer-Verlag Berlag Heidelberg, 2nd Edition. 2002;950.
5. Gupta S K: Drug Screening Methods, Jaypee publication, 1st edition, 2004: 307.
6. A.Mishra, R. Kumari, P. N. Murthy and P. P. Dash: Influence of Atorvastatin on the pharmacodynamics of Glipizide in normal and diabetic rats. Der Pharma Chemica 2010; 2 (2): 101-104.
7. Dolly Jaiswal, Prashant Kumar Rai and Geeta Watal: Antidiabetic effect of withania coagulans in experimental rats. Indian Journal of Clinical Biochemistry 2009; 24 (1): 88-93.
8. Sikarwar Mukesh S and Patil M.B: Antihyperlipidemic Effect of Ethanolic Extract of Hibiscus rosa sinensis Flowers in Hyperlipidemic Rats. RGUHS Journal of Pharmaceutical Sciences 2011; 1(2):117-122.
9. Gosh M N. Fundamentals of Experimental Pharmacology, Hilton & company, 3rd edition, 2005: 15.
10. Harish Kumar V.S., Dwajani S, Dharmveer Gurjar, Umakant patil and Vinod Kumar C.S: Effect of rosuvastatin as an anti-inflammatory agent in albino rats. Asian Journal of Pharmaceutical and Clinical Research 2011; 4(supply 1): 74-76.
11. Pyorala K, Pedersen TR, Kjekshus J, Faergeman O, Olsson AG and Thorgeirsson G: Cholesterol lowering with simvastatin improves prognosis of diabetic patients with coronary heart disease. A subgroup analysis of the scandinavian simvastatin survival Study (4S). Diabetes Care.1997 Apr; 20(4):614-20.

12. Li CB, Li XX, Chen YG, Gao HQ, Bao MC, Zhang J, Bu PL, Zhang Y and Ji XP: Simvastatin exerts cardioprotective effects and inhibits the activity of Rho-associated protein kinase in rats with metabolic syndrome. Clin Exp Pharmacol Physiol 2012 Jun 7
13. Sridevi Devaraj, Emily Chan and Ishwarlal Jialal: Direct Demonstration of an Antiinflammatory Effect of Simvastatin in Subjects with the Metabolic Syndrome. The Journal of Clinical Endocrinology & Metabolism 2006; 91: 4489-4496.
14. Bin Zhu, Hanchao Shen, Junfu Zhou, Feili Lin andYing Hu: Effects of Simvastatin on Oxidative Stress in Streptozotocin-Induced Diabetic Rats: A Role for Glomeruli Protection. Nephron Experimental Nephrology 2005; 101: e1-e8.
15. Huda E. Tawfik, Azza B. El-Remessy, Suraporn Matragoon, Guochuan Ma, Ruth B. Caldwell and R. William Caldwell: Simvastatin Improves Diabetes-Induced Coronary Endothelial Dysfunction. The Journal of Pharmacology and Experimental Therapeutics 2006; 319: 386-395.

How to cite this article:
Kumar VSH, Sindhu NR, Patil RS and Patil U: Anti-Hyperglycemic Activity of Simvastatin alone (Therapeutic Dose) and Combination of Simvastatin and Glipizide (Sub Therapeutic Doses) on Alloxan induced Hyperglycemia in Albino Rats. *Int J Pharm Sci Res*. 3(11); 4398-4403.