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Effect of a novel succinamic acid derivative as potential anti-diabetic agent in experimental diabetic rats

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ABSTRACT

4-((benzyloxy) amino)-2-hydroxy-4-oxobutanoic acid which is a succinamic acid derivative has been synthesized in 3 step reaction with malic acid. Its structure confirmation was done by various techniques like ^{1}H NMR, ^{13}C NMR, & HRMS and is recently proposed as an insulinotropic agent for the treatment of non-insulin dependent diabetes mellitus. In the present study, the effect of 4-((benzyloxy) amino)-2-hydroxy-4-oxobutanoic acid on plasma glucose, serum insulin, serum lipid profile and lipid peroxidation in streptozotocin–nicotinamide induced type 2 diabetic model was investigated. 4-((benzyloxy) amino)-2-hydroxy-4-oxobutanoic acid was administered orally (20 mg/kg b.w.) to streptozotocin + nicotinamide (STZ + NAD) induced diabetic rats for 28 days. A significant increase in fasting blood glucose levels, HbA1c levels, Serum lipid profile (TG & TC) and in the levels of Malonaldialdehyde (MDA, end product of lipid peroxidation) was observed in STZ +NAD diabetic rats whereas the levels of high density lipoprotein-cholesterol (HDL-C) and serum insulin levels were significantly decreased in STZ + NAD induced diabetic rats The effect of 4-((benzyloxy)amino)-2-hydroxy-4-oxobutanoic acid was compared with glibenclamide, a reference drug. Treatment with 4-((benzyloxy) amino)-2-hydroxy-4-oxobutanoic acid and glibenclamide resulted in a significant reduction of fasting blood glucose levels with increase in plasma insulin levels in diabetic treated rats. 4-((benzyloxy) amino)-2-hydroxy-4-oxobutanoic acid also resulted in a significant improvement in serum lipids and lipid peroxidation products. Our results suggest the potential role of 4-((benzyloxy) amino)-2-hydroxy-4-oxobutanoic acid in the management of type-2 diabetes mellitus experimental rats.

Keywords: 4-((benzyloxy) amino)-2-hydroxy-4-oxobutanoic acid, dyslipidemia, streptozotocin induced diabetes, lipid peroxidation

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INTRODUCTION

Diabetes Mellitus (DM), commonly referred as diabetes, is a group of metabolic disorders in which the sugar levels are high over a prolonged period in blood which results in hypoglycemia, lipoprotein abnormalities, raised metabolic rate. According to WHO, global prevalence of DM in 2014 was 9% among adults[1]. India is the diabetic capital of the world, predicted to have 57.2 million diabetic populations by the year 2015[2]. The estimated burden of individuals with diabetes in South East Asia aged between 20 to 79 years was equivalent to 78.3million in 2015, which was expected to rise to 140.2 million by 2040[3]. Diabetes is a progressive disease and is associated with many complications like neuropathy, retinopathy, nephropathy and cardiovascular disease.

At molecular level, insulin resistance is associated predominantly with defect in activation and expression of proximal molecules of insulin signaling pathway e.g., Insulin receptor, Insulin receptor substrate (IRS) etc.[4-5] There are many side effects associated with prolong use of insulin and hypoglycemic disease. As incident rate of diabetes mellitus continue to rise, there is growing need to identify novel antidiabetic agent with less side-effects and improved efficacy. About 80 % of world populations use the herbal drugs, for treatment of various diseases[6]. The anti-hyperglycemic activity of *Eugenia jambolana* (Botanical name- Syzgium cumini) from its seeds, fruit pulp, barkand roots has been well established[7-10].

Sharma et. al has already isolated the active antihyperglycemic compound known as alpha hydroxy succinamic acid (FIIc)(US Patent number 6,426,826 dated

6th August 2002; Indian Product Patent number. 2,30,753 February 2009) from the fruit pulp of *Eugenia jambolana*[11]. Therefore, it is expected that succinamic acid derivatives will possess antidiabetic and antioxidant properties. Alpha hydroxy acids including malic acid, glycolic acid, citric acid, tartaric acid, lactic acid and others are group of natural acids found in foods. 4-((benzyloxy) amino)-2-hydroxy-4-oxobutanoic acid is a class of alpha hydroxy acid derivative, which is widely found in food, medicine and cosmetic industries[12]. Due to the seasonal barriers and less yield of herbal anti-diabetic compound (FIIc) obtained from the fruit pulp of E.jambolana, this study was designed to synthesize and to assess the anti-hyperglycemic, hypolipidemic and antioxidant potential of 4-((benzyloxy) amino)-2-hydroxy-4-oxobutanoic acid (succinamic acid derivative) in nicotinamide-streptozotocin-induced type-2 diabetic rats. The structure of the synthesized compound is displayed in Fig. 1.

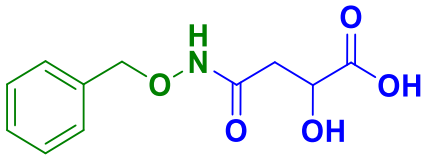


Figure 1: Structure of 4-((benzyloxy)amino)-2-hydroxy-4-oxobutanoicacid

MATERIAL AND METHODS

Chemistry

Succinamic acid derivative was synthesized according to Scheme 1 starting with malic acid. L and D form of malic acid are available as hydroxyl carboxylic acid**;** it fulfills the primary condition for selective esterification of malic acid. In order to convert malic acid to 2, the hydroxyl group present at the α position of carboxy group required for selective esterification. Malic acid was alkylated by treatment with trifluoroacetic anhydride and methanol through a malic anhydride intermediate shown in fig. 2, which was in-situ converted to give 3-hydroxy-4-methoxy-4-oxobutanoic acid(2). Compound 2 was treated with O-benzylhydroxylamine(3) using amide coupling condition to give methyl 4-((benzyloxy)amino)-2-hydroxy-4-oxobutanoate(4). Compound 5 was obtained from 4 by using basic hydrolysis conditions to give 4-((benzyloxy) amino)-2-hydroxy-4-oxobutanoic acid (5). Melting points of all synthesized compounds were determined on an electrothermal apparatus. Malic acid, TFAA, EDC. HCl, HOBT, TEA, NaOH were procured from Spectrochem Pvt. Ltd., India. All solvents were purified and dried by standard methods.

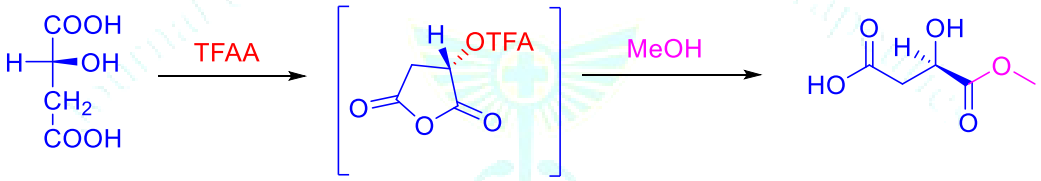
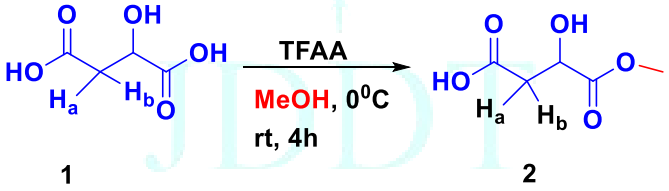


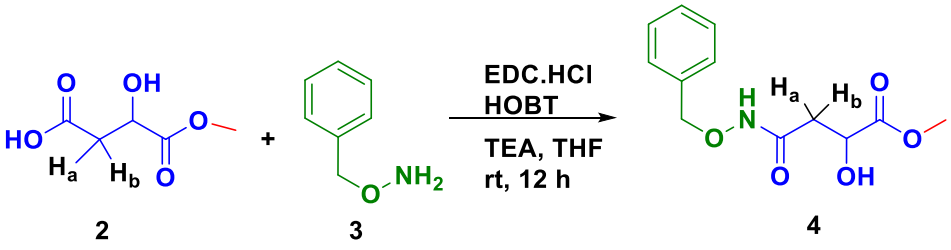
Fig. 2

Scheme: 1



Step 1:

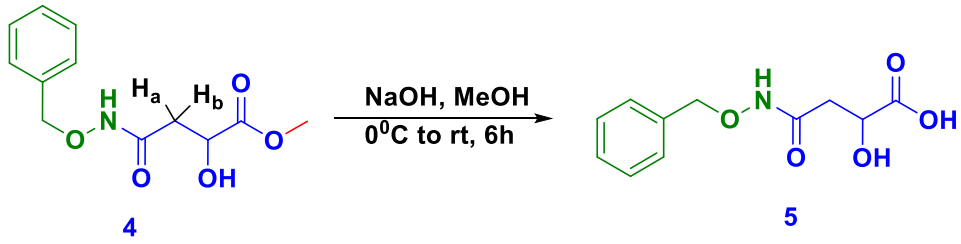
Procedure for synthesis of 3-hydroxy-4-methoxy-4-oxobutanoic acid (2) Trifluoroacetic anhydride (45 ml) was added to L-malic (1) acid (10.0 g, 1 eq) at 00C and allowed to stir at rt. After 1.5h, excess of TFAA and TFA were distilled off on rotary evaporator at temperature < 30 °C. The white crystalline compound obtained was cooled to 0°C and anhydrous methanol (50 mL) was added portion wise. The reaction mixture was further allowed to stir at rt for 3h.The progress of reaction was monitored by TLC and after the completion of reaction, excess methanol was distilled off under reduced pressure. The crude compound was purified by column chromatography using silica gel (60:120 mesh) in 10-40 % EtOAc: Hexane as solvent system. The desired compound was obtained in 40% EtOAc: Hexane as white solid. m.p. 69-70 0C ; Yield : 50.67 % ; ^{1}H NMR (400 MHz, DMSO): 12.31 (br s, ^{1}H, -COOH), 4.33 (t, ^{1}H), 3.62 (s, ^{3}H), 2.62 (d, J=15.57 Hz., ^{1}H, Ha), 2.46 (d, J=15.57 Hz., ^{1}H, Hb) ; ^{13}C NMR (100 MHz, DMSO) δ: 174.09, 172.22, 72.02, 63.67, 55.45, 36.03; HRMS (ESI) (M+H)+Calcd for $C^5H^8O^5$: 148.0372, found 148.0367.



Step 2:

Procedure for synthesis of methyl 4-((benzyloxy) amino)-2-hydroxy-4-oxobutanoate (4)

To a stirred solution of 2 (1.0 g, 1 eq) in THF, EDC.HCl (1.5 eq) was added and reaction mixture was allowed to stir at rt for 10 min. Then HOBt (1.5 eq) was added followed by the addition of TEA (3.0 eq) and compound 3 (1.2 eq). The resultant reaction mixture was allowed to stir at rt for 12 h. Progress of reaction was monitored by TLC and after the completion of reaction, it was diluted with water and extracted with EtOAc (3x50 ml). Then organic layer was washed with brine, dried over anhydrous NaSO4 and concentrated under reduced pressure. The crude product was purified by column chromatography using silica gel (60-120 mesh) in 10-40 % EtOAc: Hexane as solvent system. The desired compound was obtained in 45 % EtOAc: hexane as off white solid. m.p. 68-720C; Yield: 49.07 %; ^{1}H NMR (400 MHz, DMSO-d6): 11.05 (br s, ^{1}H, -NH),7.37-7.43 (m, 5H), 4.75 (s, ^{2}H), 4.86 (t, ^{1}H), 3.61 (s, ^{3}H), 2.35 (d, J=5.04 Hz. ^{1}H, Ha), 2.24 (d, J=7.79 Hz., Hb); ^{13}C NMR (100 MHz, DMSO) δ: 174.09, 169.22, 133.59, 129.48, 129.05, 128.46, 78.02, 63.67, 54.55, 36.03.); HRMS (ESI) (M+H)+Calcd for $C^{12}H^{15}NO^{5}$: 254.0950, found 254.1021.



4 5

Step 3:

Procedure for synthesis of 4-((benzyloxy) amino)-2-hydroxy-4-oxobutanoic acid (5)

To a stirred solution of 4 (1.0 g, 1 eq) in MeOH, aqueous solution of NaOH (5 eq) was added and allowed to stir at rt for 6 h. Progress of reaction was monitored by TLC and after completion of reaction, the reaction mixture was concentrated under reduced pressure. The reaction mixture was acidified with 1N HCl which resulted in the formation of solid compound and was filtered through sintered funnel, washed with cold H_2O and dried under high vacuum to give desired compound 5 as off white solid. m.p. 79-820C; Yield: 55.32 % ; ^{1}H NMR (400 MHz, DMSO-d6):11.03 (br s, 1H, -NH),7.39-7.32 (m, ^{5}H), 4.76 (s, ^{2}H), 4.30 (t, ^{1}H), 2.35 (dd, J=14.20 Hz. ^{1}H, Ha), 2.19 (dd, J=14.21 Hz. ^{1}H, Hb);^{13}C NMR (100 MHz, DMSO) δ: 172.62, 169.63, 134.02, 129.46, 129.04, 128.458, 78.019, 63.832, 35.972.; HRMS (ESI) (M+H)+Calcd for $C^{11}H^{13}NO^{5}$: 239.0794, found 239.0879.

Biology

Experimental animals: Male Wistar albino rats (weighing 220 - 250 grams) were procured from Central Animal House of UniversityCollege of Medical Sciences (UCMS), University of Delhi, India. The animals were housed in standard conditions of temperature (22 ± 2°C) and at 12 hour light-dark cycle. The rats were fed with commercial diet (Hindustan liver Ltd., Mumbai) and water ad libitum. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC), UCMS, Delhi, India (UCMS/IAEC/26 granted on 30th December 2013)

Induction of diabetes in rats

Overnight fasted animals were made diabetic by intra-peritoneal injection of freshly prepared Streptozotocin (Sigma Chemical Company, USA) in citrate buffer (0.1 M, pH 4.5) at a dose of 45 mg/kg body weight. Nicotinamide at a dose of 230 mg/kg body weight was given15 minutes prior to STZ injection for the development of stable type 2 diabetes mellitus[13].The control rats were only injected with citrate buffer. After 72 h of induction when blood glucose was stabilized, fasting blood glucose (FBG) was determined and rats having FBG >250 mg/dl were designated as having diabetes mellitus and were used in this experiment. The experimental period lasted for 4 weeks and day 0 was designated as the day when rats were confirmed to be diabetic.

The animals were divided into 4 groups and each group consisted of 6 rats:

- Group A : Healthy control (normal saline)
- Group B: Diabetic control (normal saline)
- Group C: Diabetic treated with 4-((benzyloxy)amino)-2-hydroxy-4-oxobutanoic acid
- Group D: Diabetic treated with glibenclamide

1/50 of LD_{50} was considered as sublethal dose of 4-((benzyloxy) amino)-2-hydroxy-4-oxobutanoic acid and it was used as therapeutic dose in the subsequent work which was calculated to be 18 mg/kg b.w. Glibenclamide was given as a standard drug orally at a dose of 600µg/kg of body weight / day for 4 weeks to group D.

Acute toxicity study and determination of LD_{50}

LD_{50} of the studied compound 4-((benzyloxy) amino)-2-hydroxy-4-oxobutanoic acid was determined as described by Afifi et al[14]. In this experiment, six groups each of 6 male albino rats weighing 180-220 g were used. One group serves as control andother groups of mice were orally administered the tested compound by gastric tube in gradual increasing doses (200, 400, 600, 800 and 1000mg/ kg b. w.). After 48 hours of administration, the number of dead animals in each group was counted, mean of dead animals in two successive doses (z) and the constant factor between two successive doses (d) were recorded and LD_{50} was calculated as follow:

LD_{50} = the highest dose which kill all animals - Σ(z.d)/n

Where n: number of animals in groups = six animals in eachgroup.

Biochemical parameters:

Blood was drawn from retro orbital plexus by using micro-capillary technique from all overnight fasted animals on

day 1 and afterwards at week 4 of the study. Whole blood was drawn for the estimation of glycosylated hemoglobin and plasma/serum was separated from blood for the estimation of fasting blood glucose, lipid profile, serum insulin levels and oxidative stress parameters. These samples were carefully processed and stored in -80 °C deep freezer. All the parameters were measured using commercially available kits: Plasma fasting blood glucose (Centronic, GmbH, Gemany), Glycosylated Hemoglobin (Hb1Ac; Biosystems S.A., Costa Brava, Spain), Total serum cholesterol (Infinite; Accurex Biomedical, Thane, India), Serum triglycerides (Infinite; Accurex Biomedical, Thane, India), HDL-Cholesterol (Infinite; Accurex Biomedical, Thane, India) Insulin (Ray Biotech Rat ELISA kit, USA) and Malondialdehyde (MDA) levels using standard techniques.

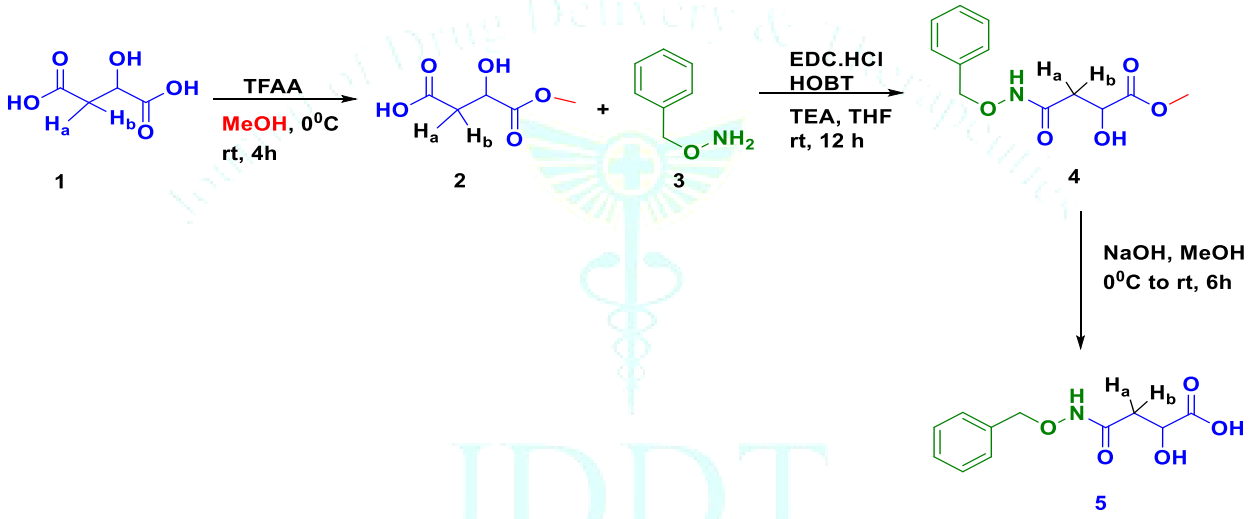
Insulin test was performed using Rayto 2100c microplate ELISA reader (Rayto, China). The amount of insulin was quantified by sandwich enzyme-linked immunosorbent assay (ELISA). The absorbance was measured at 450 nm through ELISA plate reader

Statistical analysis: Two ways ANOVA was applied for the comparison of parameters between the groups followed by Tukey's test. Pearson's coefficient of correlation was calculated for all the 4 groups together and separately for all the above mentioned parameters. Difference was assumed to be significant at the level of $p < 0.05$.

RESULTS & DISCUSSION

Chemistry (Synthesis)

In this research work, we have synthesized derivative of hydroxy succinamic acid with one polar and other side non polar as building block for preparation of α-hydroxy acid.3 Our synthetic strategy starts from the easily available compound, malic acid and trifluoroacetiticanhydride, which converted into cyclic anhydride intermediate, then this cyclic anhydride intermediate on treatment with MeOH led to the synthesis of 3-hydroxy-4-methoxy-4-oxobutanoic (2). Then, this compound was treated with O-benzylhydroxylamine followed by the amide coupling condition to give methyl 4-((benzyloxy) amino)-2-hydroxy-4-oxobutanoate (4).Then compound (4) was hydrolyzed under basic conditions to give target compound 4-((benzyloxy)amino)-2-hydroxy-4-oxobutanoic acid (5). Target compound (5) was synthesized. All synthesized compound confirmed by ^{1}H-NMR, ^{13}C-NMR and HRMS data.



Biological studies

For determination of lethal dose LD_{50} of 4-((benzyloxy)amino)-2-hydroxy-4-oxobutanoic acid, single gradual increasing doses were administered to various groups of normal albino rats. The number of dead animals in each group was counted after 48 hours of compound administration and LD_{50} was calculated which was found to be 767 mg/kg b.w. Based on this toxicity study, the orally therapeutic dose was calculated (18 mg/kg of b.w.) which is about1/50 of LD_{50} which is so far from LD_{50}. (**Table 1**)

In the present study, a significant improvement was observed in glycemic index, serum insulin, lipid profile and lipid peroxidation products in 4-((benzyloxy) amino)-2-hydroxy-4-oxobutanoic acid treated rats. The various biochemical parameters has been summarized in **Table 2 & 3**

Table 1: Determination of LD_{50} of **4-((benzyloxy) amino)-2-hydroxy-4-oxobutanoic acid** in male albino wistar rats

Dose (mg/kg b.w.)	Total no of animals	No of dead animals	z	d	∑(z.d)
200	6	0	-	200	-
400	6	1	0.5	200	100
600	6	2	1.5	200	300
800	6	3	2.5	200	500
1000	6	4	3.5	200	700
1200	6	6	5	200	1000

z: mean number of dead animals in two successive doses
d: constant factor between two successive doses
LD_{50} = Median lethal dose which kill all animals - Σ(z.d)/n = 1200-2600/6 =767mg /kg b. w.

1/50 of LD_{50} is about 18 mg /kg b. w. which was considered as sublethal dose that was used as therapeutic dose in the subsequent studies.

Table 2: Showing glycemic index and serum insulin levels at week 0 and at week 4 after treatment with 4-((benzyloxy) amino)-2-hydroxy-4-oxobutanoic acid and glibenclamide

Parameters	Time points	Group A	Group B	Group C	Group D
FBG (mg/dl)	Week 0	97±6.7	233.2 ± 7.9 [a]	226 ±7.85[b,d]	222.4 ±9.6[c,b]
	Week 4	96±5.64	247.45± 5.64[a]	124.3± 5.46[b,d]	118+2.75[c,b]
HbA1c %	Week 0	5.01±0.12	5.32±0.28[a]	5.24±0.29[b,d]	5.38±0.22[c,b]
	Week 4	5.18±0.10	8.58±0.68[a]	6.01±0.22[b,d]	5.94±0.23[c,b]
Serum Insulin (pmol/L)	Week 0	15.16±0.64	8.86±0.58[a]	8.96±0.34[b,d]	8.67±0.24[c,b]
	Week 4	15.64±0.56	7.46±0.19[a]	12.46±0.42[b,d]	13.12±0.35[c,b]

Values are mean ± S.D. (n=6) (p<0.001)
a= Group A vs Group B, b= Group A vs Group C, c= Group A vs Group D, d= Group B vs Group C

Effect of 4-((benzyloxy) amino)-2-hydroxy-4-oxobutanoic acid on fasting blood glucose levels

FBG levels were measured at week 0 & week 4 for entire experimental groups. A significant (p<0.01) decrease in FBG levels were observed in **4-((benzyloxy) amino)-2-hydroxy-4-oxobutanoic acid** and glibenclamide treated groups as compared to diabetic control rats.

Effect of 4-((benzyloxy) amino)-2-hydroxy-4-oxobutanoic acid on HbA1c levels

The glycosylated Hb (HbA1c) level was significantly increased in the diabetic control rats when compared to normal control (p<0.001). The HbA1c level was lowered significantly in **4-((benzyloxy) amino)-2-hydroxy-4-oxobutanoic acid** and glibenclamide treated groups.

Effect of 4-((benzyloxy) amino)-2-hydroxy-4-oxobutanoic acid on serum Insulin levels

The serum insulin levels in the diabetic control rats was found to be 8.86±0.58 pmol/L which was significantly decreased (p<0.001) when compared to normal rats. However, a significant increase (p<0.01) in serum insulin levels in **4-((benzyloxy) amino)-2-hydroxy-4-oxobutanoic acid** and glibenclamide treated groups was observed.

Table 3: Showing serum lipids and malonaldialdyhde (MDA) levels at week 0 and at week 4 after treatment with **4-((benzyloxy) amino)-2-hydroxy-4-oxobutanoic acid** and glibenclamide

Parameters	Time points	Group A	Group B	Group C	Group D
Serum Total Cholesterol (TC) (mg/dl)	Week 0	60.55±4.48	61.23±7.66[a]	62.8±5.38 [b,d]	60.04±6.34 [c,b]
	Week 4	61.92±4.32	90.86±6.48[a]	72.04±6.09 [b,d]	76.04±4.09 [c,b]
Serum Triglycerides (TG) (mg/dl)	Week 0	63.8±5.01	65.60±4.45[a]	64.14±4.8 [b,d]	67±6.62 [c,b]
	Week 4	65.8±4.14	104.0±6.26[a]	82.26±5.01 [b,d]	86.34±5.01 [c,b]
HDL-Cholesterol (HDL-c) (mg/dl)	Week 0	38.2±1.68	37.32±1.60[a]	37.67±1.50 [b,d]	37.33±1.63 [c,b]
	Week 4	37.67±1.09	25.17±1.12[a]	34.80±1.09 [b,d]	32.80±1.16 [c,b]
Malondialdehyde (MDA) (pmol/mg)	Week 0	4.2±0.42	4.1±0.25[a]	4.32±0.22 [b,d]	4.1±0.36 [c,b]
	Week 4	4.3±0.26	8.08±0.45[a]	6.4±0.15 [b,d]	5.92±0.48 [c,b]

Values are mean ± S.D. (n=6) (p<0.001)
a= Group A vs Group B, b= Group A vs Group C, c= Group A vs Group D, d= Group B vs Group C

Effect of 4-((benzyloxy) amino)-2-hydroxy-4-oxobutanoic acid on serum TC

The mean serum total cholesterol levels of normal control rats was 60.56 ± 4.48 mg/dl, which was significantly (p < 0.001) increased to 90.86 ± 6.48 mg/dl in the diabetic control rats (Table 3). This increased serum TC level was significantly decreased by treatment with 4-((benzyloxy)amino)-2-hydroxy-4-oxobutanoic acid and glibenclamide

Effect of 4-((benzyloxy) amino)-2-hydroxy-4-oxobutanoic acid on serum TG

The mean serum triglyceride level of normal control rats was 64.80 ± 4.15 mg/dl, which significantly (p < 0.001) increased to 104.00 ± 6.26 mg/dl in the diabetic control rats (Table 3). This increased serum triglyceride level significantly decreased by treatment with 4-((benzyloxy) amino)-2-hydroxy-4-oxobutanoic acid and glibenclamide.

Effect of 4-((benzyloxy) amino)-2-hydroxy-4-oxobutanoic acid on serum HDL-c

Induction of diabetes caused significant (p < 0.001) decrease in serum HDL-cholesterol levels of 38.20± 81.68 mg/dl to 25.17 ± 1.12 mg/dl when compared against normal control rats (Table 3). Treatment with 4-((benzyloxy) amino)-2-hydroxy -4-oxobutanoic acid produced significant increase in the serum HDL-cholesterol levels.

Effect of 4-((benzyloxy) amino)-2-hydroxy-4-oxobutanoic acid on Malondialdehyde levels

Compared to normal control rats, diabetic control rats showed a significant increase in MDA levels (p<0.001). 4-((benzyloxy)amino)-2-hydroxy-4-oxobutanoic acid and glibenclamide treated groups showed a significant decrease in the MDA levels when compared to diabetic control rats (p<0.001).

In our study, we have observed that 4((benzyloxy)amino)-2-hydroxy-4-oxobutanoic acid) decreases plasma glucose and increased plasma insulin in streptozotocin–nicotinamide induced diabetic rats. The possible mechanism of action of 4((benzyloxy)amino)-2-hydroxy-4-oxobutanoic acid) that can be correlated with the effect of

sulphonylureas that promote insulin secretion by closure of K+- ATP channels, membrane depolarization and stimulation of Ca^{2+} influx, an initial key step in insulin secretion[15-16].

In diabetes hyerpglycemia is accompanied with dyslipidemia i.e., characterized by increase in TC, TG & fall in HDL-c. The increased serum lipids (TG & TC) which may be due to due to the increased mobilization of free fatty acids from peripheral deposits, since insulin inhibits hormone sensitive lipase[17]. This altered serum lipid profile was significantly ($p<0.001$) revered back to normal after treatment with 4((benzyloxy) amino)-2-hydroxy-4-oxobutanoic acid). This suggests its potential as a lipid lowering agent.

It has been found that the rate of formation of Malonaldialdehyde (MDA) is significantly increased in diabetic rats compared to healthy rats[18]. Several studies have confirmed the involvement of free radicals in the genesis of diabetes mellitus and their role in the induction of lipid peroxidation during diabetes[19]. The possible mechanism that can be correlated would be the diffusion of lipid peroxidation products from the site of tissue damage and therefore can be measured in plasma[20].

Our findings indicate that 4((benzyloxy) amino)-2-hydroxy-4-oxobutanoic acid) possesses potent antioxidant properties due to its inhibition of the formation of lipid peroxidation end-product, MDA, in the type 2 diabetic rats.

CONCLUSION

In this study, we have found that the novel synthetic 4-((benzyloxy)amino)-2-hydroxy-4-oxobutanoic acid has anti-hyperglycemic, hypolipidemic and antioxidant potentials in STZ +NAD induced type 2 diabetic rats. These effects may be due to insulinogenic action and extrapancreatic effects in addition to the enhancing action on the antioxidant defense system. However, further clinical studies are required to assess the safety and efficacy of the of 4-((benzyloxy) amino)-2-hydroxy-4-oxobutanoic acid and to elucidate its role a potent antidiabetic agent.

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Conflicts of interest:

The authors declare that they have no conflicting interest.

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