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Coagulanolide, a withanolide from *Withania coagulans* fruits and antihyperglycemic activity ☆

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ABSTRACT

One new withanolide, (17*S*,20*S*,22*R*)-14α,15α,17β,20β-tetrahydroxy-1-oxowitha-2,5,24-trienolide) named coagulanolide (**4**) along with four known withanolides **1**–**3** and **5** have been isolated from *Withania coagulans* fruits and their structures were elucidated by spectroscopic techniques. The compounds **1**–**5** showed significant inhibition on postprandial rise in hyperglycemia post-sucrose load in normoglycemic rats as well as streptozotocin-induced diabetic rats. The compound **5** also showed significant fall on fasting blood glucose profile and improved the glucose tolerance of db/db mice. Further compound **5** showed antidyslipidemic activity in db/db mice. The median effective dose of the compound **5** was determined to be around 25 mg/kg in streptozotocin-induced diabetic rats, which is comparable to the standard antidiabetic drug metformin. Our results provide further support to explain the traditional use of *W. coagulans* as antihyperglycemic cum antidyslipidemic agent by the traditional medical practitioners.

Withania coagulans Dunal (Family: Solanaceae) is popularly known as Indian cheese maker. The aqueous extract and 3-β-hydroxy-2,3-dihydrowithanolide F of fruits of this plant has been shown to exert hepatoprotective activity against CCl_4 induced hepatotoxicity in adult albino rats.[1] The alcoholic extract and total alkaloids also showed significant anti-inflammatory effect in acute inflammation induced with egg albumin, sub-acute inflammation induced with formalin and granulation tissue formation by cotton pellet method.[2] Oral administration of aqueous extract of fruits of *W. coagulans* (1.0 g/kg) was earlier reported to lower the blood sugar, serum cholesterol, serum LPO and hepatic LPO levels in normal as well as streptozotocin-induced diabetic rats.[3] The aqueous extract also exhibited free radical scavenging activity in an in vitro system using DPPH.[3] It also showed hypolipidemic activity in triton induced hypercholesterolemic rats.[4] Antifungal and antibacterial properties have also been demonstrated in the withanolides isolated from ethanolic extract of the whole plant and leaves, respectively.[5,6] The volatile oil obtained from fruits of *W. coagulans* had antibacterial activity against *S. aureus* and *Vibrio cholerae* and also found to have antihelminthic activity.[7] Cardiovascular effects of withanolide isolated from *W. coagulans* fruits have been reported.[8] The extract has hypotensive, respiratory stimulant and smooth muscle relaxant activity in experimental animals.[9]

Previous chemical investigation of this plant resulted in the isolation and identification of several withanolides.[5,10–12] The effective treatment of diabetes is increasingly dependent on active constituents of medicinal plants capable of controlling hyperglycemia as well as its secondary complications. The natural active principles of *W. coagulans* contributing to antihyperglycemic activity were not determined. It was therefore, necessary to determine the active antihyperglycemic agent, if any present in the fruits of *W. coagulans*, particularly in its aqueous decoctions, which is commonly used in the traditional system of medicine. We also demonstrated significant antihyperglycemic activity in the aqueous extract of *W. coagulans* fruits when evaluated in normoglycemic as well as in STZ-induced diabetic rat models at 250 mg/kg po dose level. In order to isolate the major active principles, the aqueous extract of *W. coagulans* was subjected to extensive column chromatographic procedure.[13] This process led to the isolation of five withanolides identified as coagulin C[14] (**1**), 17β-hydroxywithanolide K[5,10] (**2**), withanolide F[15] (3) isolated for the first time from this plant, (17*S*,20*S*,22*R*)-14α,15α,17β,20β-tetrahydroxy-1-oxowitha-2,5,24-trienolide (**4**)[16] is a new compound and coagulin L[17] (14*R*,17*S*,20*S*,22*R*)-14,17,20-trihydroxy-3β-(*O*-β-D-glucopyranosyl)-1-oxowitha-5,24-dienolide (**5**), is known in this plant, shown in Figure 1.

The structure of compound **4** was elucidated using spectroscopic techniques. Compounds **1**–**3** and **5** were characterized by

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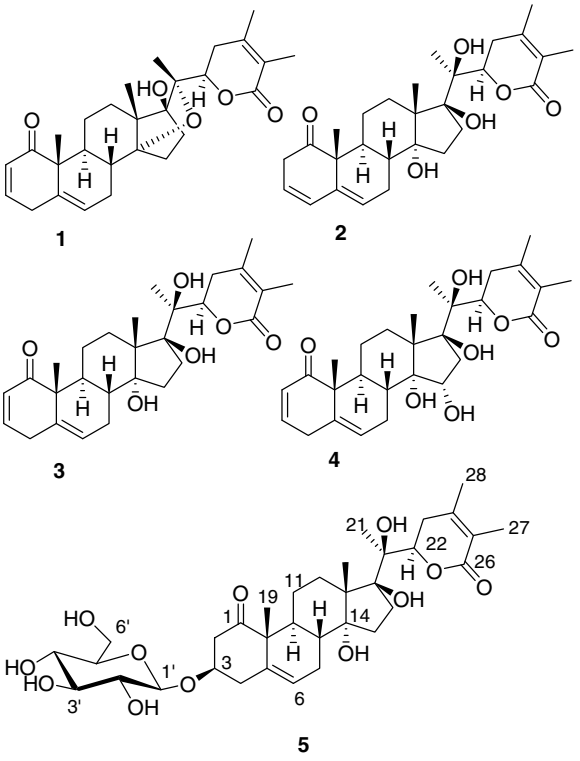

Figure 1. Isolated compounds.

Table 1
^{1}H and ^{13}C NMR spectral data of compound **4**

Position	^{1}H NMR (δ) coupling constants (J in Hz)	^{13}C NMR (δ)
1	–	204.5
2	5.87 dd (10.1, 2.1)	128.3
3	6.77 m	145.4
4	3.30 d ($J4\alpha,4\beta$ = 21.6)	33.7
	2.83 dd ($J4\alpha,4\beta$ = 21.6, $J3,4\beta$ = 4.8)	
5	–	135.3
6	5.60 br d (6.1)	125.2
7	1.98 m, 1.90 m	32.3
8	1.52 m	34.4
9	1.60 m	35.9
10	–	53.7
11	1.50 m	23.2
12	1.69 m	24.9
13	–	53.7
14	–	82.6
15	3.98 d (6.3)	74.3
16	3.07 dd (16.2, 6.3)	47.6
	1.54 d (16.2)	
17	–	88.5
18	1.13 s	20.0
19	1.23 s	18.9
20	–	78.9
21	1.43 s	20.8
22	4.94 dd ($J22,23\alpha$ = 11.8, $J22,23\beta$ = 3.5)	80.9
23	2.53 m, 2.21 m	33.4
24	–	151.5
25	–	121.2
26	–	167.3
27	1.88 s	12.5
28	1.94 s	20.9

comparison with reported spectroscopic data. In this paper we report the isolation, structure elucidation of new compound **4** and antihyperglycemic activity of compounds **1**–**5**.

Compound **4** was isolated as an amorphous powder. The FAB-mass and HRESIMS spectra showed peaks at m/z 509 $[M+Na]^+$ and 486.2611 $[M]^+$ respectively, corresponding to the molecular formula $C_{28}H_{38}O_7$. This conclusion was supported by the ^{13}C NMR and DEPT spectra. It displayed diagnostic spectral features of typical withanolide; a base peak at m/z 125 formed by cleavage across the C-20/C-22 bond, confirmed the presence of a six-membered lactone moiety and ion at m/z 169 obtained by the cleavage of C-17/C-20 bond, indicated the presence of 20-hydroxy, in its ESIMS.[18] The UV spectrum (MeOH) of **4** showed a strong absorption at 218 nm, which is characteristic of α,β-unsaturated carbonyl and α,β-unsaturated lactone chromophores.[19] The IR spectrum (KBr) displayed bands at 3426, 1712 and 1684 cm^{-1} indicative of hydroxyl, α,β-unsaturated lactone and six-membered cyclic ketone functionalities, respectively. ^{13}C NMR data (Table 1) of compound **4** revealed 28 carbon signals that were assigned by DEPT analysis to five methyls (δ 12.5, 18.9, 20.0, 20.8 and 20.9), six methylenes (δ 47.6, 33.7, 33.4, 32.3, 24.9 and 23.2), seven methines (two CH at δ 34.4 and 35.9, two CH–O– at δ 74.3 and 80.9, and three olefinic carbons at δ 125.2, 128.3 and 145.4), ten quaternary carbons (two at δ 53.7, three olefinic carbons at δ 121.2, 135.3 and 151.5, three oxygenated at δ 78.9, 82.6 and 88.5, two carbonyls at δ 167.3 and 204.5).

The ^{1}H NMR spectrum (Table 1) showed three singlets at δ 1.13, 1.23 and 1.43 which were assigned to the C-18, C-19 and C-21 methyl groups, respectively. The chemical shift of the deshielded C-21 methyl singlet and the appearance of the C-22 methine proton signal as double doublet at δ 4.94 (J = 11.8, 3.5 Hz) also indicated the presence of a hydroxy group at C-20. Further the H-22 showed correlation with C-20 (δ 78.9), C-21 (δ 20.8), C-23 (δ 33.4) and C-24 (δ 151.5) in the HMBC spectrum. Stereochemistry at C-22 is R as found in all withanolides is of biogenetic reason.[17] The downfield chemical shifts at δ 1.88 and 1.94 for C-27 and C-28 methyl singlets, respectively, indicated that they both are substituted on the double bond of conjugated lactone group in the side chain. The three downfield signals at δ 5.87 (dd, J = 10.1, 2.1 Hz), 6.77 (m) and 5.60 (br d, J = 6.1 Hz) represented three olefinic protons H-2, H-3 and H-6, respectively. The splitting pattern of the allylic methylene signals at δ 3.30 (d, J = 21.6 Hz) and 2.83 (dd, J = 21.6, 4.8 Hz) indicated that C-4 is unsubstituted and that the vicinal C-5 bears no hydrogen atom. The doublet at δ 3.98 (J = 6.3 Hz) was assigned to proton at C-15. The down field shift indicated that hydroxyl group can be placed at C-15. The orientation of secondary OH group at C-15 was assumed to be α based on the fact that the coupling pattern (J = 6.3 Hz) similar to the pattern observed in coagulin H.[17] The 15-methine proton showed interaction with the C-18 methyl group in the nuclear overhauser effect (NOE) difference spectra. This is in favour of 15α-OH group. In the HMBC spectrum H-15 showed two- and three-bond couplings to C-14 (δ 82.6), C-13 (δ 53.7) and C-17 (δ 88.5). The H-15 (δ 3.98) exhibited strong-cross-peaks with one methylenic proton H-16 at δ 3.07 in COSY 45° spectrum of **4**. Further in the COSY 45° spectrum, the C-22 methine proton (δ 4.94) showed vicinal coupling with the C-23 methylene protons (δ 2.53, 2.21). The H-3 (δ 6.77) showed connectivities with the H-2 (δ 5.87) and H-4 methylene (δ 3.30 and 2.83). The H-6 (δ 5.60) showed cross-peaks with H-7 methylene. Further the functional groups assignments were achieved by heteronuclear single quantum coherence (HSQC) and heteronuclear multiple bond connectivity (HMBC) experiments. In the HSQC spectrum, the C-15 methine (δ 74.3) was coupled with the proton at δ 3.98, while the C-22 (δ 80.9) showed coupling with the proton at δ 4.94. The C-2, C-3 and C-6 methines (δ 128.3, 145.4 and 125.2) showed cross-peaks with H-2, H-3 and H-6 at δ 5.87, 6.77 and 5.60, respectively. The C-4, C-7, C-11, C-12, C-16 and C-23 methylenes resonating at δ 33.7, 32.3, 23.2, 24.9, 47.6 and 33.4 exhibited cross-peaks with the H-4, H-7, H-11, H-12, H-16 and H-23 at (δ 3.30, 2.83), (δ 1.98, 1.90), (δ 1.50), (δ 1.69),

(δ 3.07, 1.54), and (δ 2.53, 2.21), respectively. Stereochemistry at position 14, 17 and 20 is defined on comparing the spectra with that of known withanolides.[17]

The ^{1}H and ^{13}C NMR of compound **4** showed that it had close resemblance in substitution pattern of rings A, B and C with compound **3**, the difference being the presence of a hydroxyl group at C-15. On the basis of these spectroscopic evidences led to the structure (17*S*,20*S*,22*R*)-14α,15α,17β,20β-tetrahydroxy-1-oxowitha-2,5,24-trienolide (**4**) for this new withanolide, named coagulanolide (Fig. 1).

The compounds were evaluated for their antihyperglycemic activity in normoglycemic rat model (SLM) and in streptozotocin-induced diabetic rat model (STZ) as well as in a well characterized model of type 2 diabetes, that is, C57BL/KsJ-db/db mice.

Most of the compounds were evaluated for in vivo antihyperglycemic activity in normoglycemic rat model (SLM) and STZ-induced β-cell damaged diabetic male Sprague–Dawley rats[20–22] and activities were compared with standard drug metformin. The most active compound **5** was found to improve glucose tolerance up to the tune of 29.8% in SLM and 23.3% in STZ-induced diabetic rats at a dose of 100 mg/kg body weight. Besides, compound **1**, **2**, **3** and **4** exhibited significant antihyperglycemic activity, 22.8%, 20.4%, 24.9% and 28.1% in SLM and 16.9%, 15.8%, 18.2% and 19.3% in STZ, models, respectively (Table 2). Following the confirmation of antihyperglycemic activity in STZ-induced β-cell damaged diabetic rats. The most active compound **5** was further evaluated in db/db mice.

The C57BL/KsJ-db/db mice at 12 weeks of age exhibited most of the human characteristics of type 2 diabetes including hyperglycemia in the fasting and fed states, hyperinsulinemia and insulin resistance, as already reported.[23] The db/db mice supplemented with compound **5** at a dose of 50 mg/kg body weight for 10 consecutive days, significantly lowered the postprandial blood glucose level by 22.7% ($p < 0.01$), whereas reference standard drug metformin declined the postprandial blood glucose by 18.6% ($p < 0.05$), when compared to vehicle-treated control group (Table 3).[24]

Prior to treatment db/db mice demonstrated basal hyperglycemia and this hyperglycemia was exacerbated by the oral glucose load, and failed to return to fasting level after 120 min, indicating glucose intolerance (data not shown). After 10 days of treatment, the glucose tolerance significantly improved in the compound **5** treated group, compared to the vehicle-treated group (Fig. 2). It was observed that significant ($p < 0.05$) inhibition in rise of postprandial blood glucose level at time interval 90 min and 120 min in compound **5** as well as metformin treated group. For the compound **5** db/db mice group, the area under the curve (AUC) of blood glucose decreased by approximately 40.5% compared to vehicle-treated group. In a similar experiment metformin showed 24.8% reduction in AUC of db/db mice.

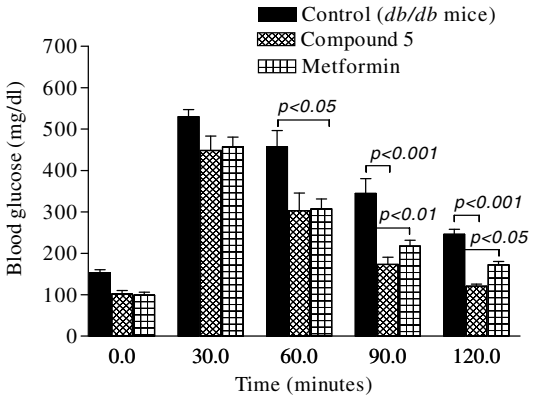

Figure 2. Oral glucose tolerance test of db/db mice treated with compound **5** and metformin at a dose of 50 mg/kg of body weight. Values are expressed as mean ± SD, $N = 5$, $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ vs control at different time intervals.

In the other experiment, compound **5** at a dose of 50 mg/kg body weight, showed significant improvement in plasma lipid profiles of dyslipidemic db/db mice after 10 days of consecutive treatment (Table 4). The treatment with compound **5** lowered the level of plasma triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C), respectively. Whereas, rise in high-density lipoprotein cholesterol (HDL-C) level was recorded in the treated group compared to control group (Table 4). Results were compared with standard drug fenofibrate.

The median effective dose (ED_{50}) of compound **5** was determined in streptozotocin-induced rats. The effect of increasing oral doses of compound **5** declined the postprandial glucose level, was dose-dependent with a calculated ED_{50} of around 25 mg/kg of body weight following oral administration.

In conclusion, our study demonstrates the isolation and characterization of biologically active withasteroids. All the compounds **1**–**5** showed significant inhibition on postprandial rise in hyperglycemia post-sucrose load in normoglycemic rats and in streptozotocin-induced diabetic rats. The compound **5** showed significant fall in peripheral blood glucose profile and also improved the glucose

Table 2
Antihyperglycemic activity of compounds **1**–**5** in normoglycemic and STZ-induced diabetic rats

Compounds	Dose (mg/kg)	% Antihyperglycemic activity	
		SLM-model	STZ-model
1	100	22.8*	16.9*
2	100	20.4*	15.8*
3	100	24.9**	18.2**
4	100	28.1**	14.7
5	100	29.8**	23.3**
Metformin	100	26.4**	17.1*

Values are expressed as mean ± SEM, $N = 5$, $^{*}p < 0.05$ and $^{**}p < 0.01$ vs vehicle-treated control.

Table 3
Antihyperglycemic effect of compound **5** and metformin in C57BL/KsJ-db/db mice post 10 days treatment

Compounds	Test dose (mg/kg)	% cure in postprandial blood glucose level	% improvement in OGTT
5	50	22.7**	44.1***
Metformin	50	18.6*	24.8**

Values are expressed as mean ± SEM, $N = 5$, $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ vs control.

Table 4
Effect of compound **5** on the plasma lipid profile in C57BL/KsJ-db/db mice

Lipid profiles of db/db mice	Control (1% gum acacia)	Compound **5** (50 mg/kg)	Fenofibrate (50 mg/kg)
TG (mg/dl)	140.8 ± 6.07	120.0 ± 4.48 (−14.7%)	118.0 ± 4.02 (−16.2%)*
CHOL (mg/dl)	160.4 ± 5.17	119.1 ± 2.01 (−25.7%)**	110.9 ± 3.71 (−30.8%)**
HDL-C (mg/dl)	56.6 ± 2.52	70.6 ± 3.50 (+24.7%)**	63.6 ± 4.01 (+12.4%)
LDL-C (mg/dl)	146.3 ± 4.28	115.2 ± 2.13 (−21.2%)**	110.5 ± 4.11 (−24.5%)**
VLDL-C (mg/dl)	28.2 ± 0.56	23.8 ± 0.34 (−15.6%)*	22.1 ± 0.19 (−21.6%)**

Values are expressed as mean ± SEM, $N = 5$, $^{*}p < 0.05$ and $^{**}p < 0.01$ vs control. Statistical analysis was done by student's *t*-test.
– denotes decrease in parameter compared to vehicle-treated control group.
+ denotes increase in parameter compared to vehicle-treated control group.

tolerance of db/db mice. Further compound **5** also showed antidyslipidemic activity in db/db mice that is comparable to median effective dose of fenofibrate, that is, 50 mg/kg body weight. The median effective dose of the compound **5** was determined to be around 25 mg/kg in streptozotocin-induced diabetic rats, which is better than the standard drug metformin. Beside this, the compound **5** also showed antidyslipidemic activity in db/db mice. Thus, compound **5** can be developed as an antidiabetic lead molecule and further studies needs to be carried out in this direction to find out the mechanism of action of this molecule.

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References and notes

1. Budhiraja, R. D.; Sudhir, S.; Craeg, F. N.; Arora, B. *Planta Med.* **1986**, *1*, 28.
2. Budhiraja, R. D.; Sudhir, S.; Garg, K. N. *Planta Med.* **1977**, *32*, 154.
3. Hemalatha, S.; Wahi, A. K.; Singh, P. N.; Chansuria, J. P. N. *J. Ethnopharmacol.* **2004**, *93*, 261.
4. Hemalatha, S.; Wahi, A. K.; Singh, P. N.; Chansuria, J. P. N. *Phytother. Res.* **2006**, *20*, 614.
5. Choudhary, M. I.; Shahwar, Dur-E.; Zeba, P.; Jabbar, A.; Ali, I.; Rahman, Atta-Ur. *Phytochemistry* **1995**, *40*, 1243.
6. Khan, M. T. J.; Ashraf, M.; Tehniyat, S.; Bukhtair, M. K.; Ashraf, S.; Ahmed, W. *Fitoterapia* **1993**, *64*, 367.
7. Gaind, K. N.; Budhiraja, R. D. *Indian J. Pharm.* **1967**, *29*, 185.
8. Budhiraja, R. D.; Sudhir, S.; Garg, K. N. *Indian J. Physiol. Pharmacol.* **1983**, *27*, 129.
9. Siddiqui, H. H.; Israili, A. H.; Qadry, S. M. J. S. *Planta Med.* **1963**, *2*, 145.
10. Rahman, Atta-Ur.; Choudhary, M. I. *Pure Appl. Chem.* **1998**, *70*, 385.
11. Vincent, V. V.; David, L.; Budhiraja, R. D.; Sharan, S.; Garg, K. N. *Phytochemistry* **1983**, *22*, 2253.
12. Neogi, P.; Kawai, M.; Butsugan, Y.; Mori, Y.; Suzuki, M. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 4479.
13. The dried fruits of *W. coagulans* (1 kg) were boiled with distilled water repeatedly (4 times). The combined aqueous extract was filtered and concentrated under reduced pressure using rotavapour at 45 °C to a brown gum (123.0 g). An aliquot of the extract (100.0 g) was dissolved in minimum water H_2O (500 ml), extracted successively with hexane and ethyl acetate. The ethyl acetate soluble fraction (15.0 g) was subjected to repeated column chromatography over flash silica gel. Compound **1** (81 mg) was obtained from $CHCl_3$–MeOH (99:1) eluate. Compound **2** (90 mg), **3** (105 mg) and **4** (97 mg) were obtained from $CHCl_3$–MeOH (19:1) eluate. The aqueous fraction was reduced to around 100 ml using rotavapour at 45 °C and subjected to HP 20 Diaion resin (LabION) (1.0 kg) column. The column was eluted with H_2O, H_2O:MeOH (1:1 and 1:4), and then MeOH. A fraction obtained, elution with MeOH was subjected to repeated column chromatography on flash silica gel. The compound 5 (1.5 g) was obtained from $CHCl_3$–MeOH (17:3) eluate. The extraction and isolation procedure was repeated with fruits of *W. coagulans* (5.0 kg) to get the enough compounds for activity testing.
14. Rahman, Atta-Ur.; Shabbir, M.; Shahwar, Dur-E.; Choudhary, M. I.; Voelter, W.; Hohnholz, D. *Heterocycles* **1998**, *47*, 1005.
15. Glotter, E.; Abraham, A.; Gunzberg, G.; Kirson, I. *J. Chem. Soc., Perkin Trans. I* **1977**, 341.
16. (17*S*,20*S*,22*R*)-14α,15α,17β,20β-Tetrahydroxy-1-oxo -witha-2,5,24-trienolide (**4**). White amorphous powder, $[\alpha]_D$ +54° (*c* = 0.2, MeOH); IR ν_{max} (KBr) cm^{-1}: 3426, 1712 and 1684; UV λ_{max} (MeOH) nm: 218; FABMS: *m*/*z* 509 $[M+Na]^+$; ESIMS *m*/*z* 486 $[M]^+$, 169, 125; HRESIMS *m*/*z* 486.2611 $[M]^+$; ^1H and ^{13}C NMR data are in Table 1.
17. Rahman, Atta-Ur.; Yousaf, M.; Gul, W.; Qureshi, S.; Choudhary, M. I.; Voelter, W.; Hoff, A.; Jens, F.; Naz, A. *Heterocycles* **1998**, *48*, 1801.
18. Subramanian, S. S.; Sethi, P. D.; Glotter, E.; Kirson, I.; Lavie, D. *Phytochemistry* **1971**, *10*, 685.
19. Lavie, D.; Glotter, E.; Shvo, Y. *J. Org. Chem.* **1965**, *30*, 1774.
20. Shukla, P.; Singh, A. B.; Srivastava, A. K.; Pratap, R. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 799.
21. *Normoglycemic rats.* Male albino rats of Sprague–Dawley strain (body weight 140 ± 20g) were selected for this study. Fasting blood glucose of each animal (14–16 h starvation) was measured and the animals showing blood glucose level between 60 and 80 mg/dl were finally selected and divided into groups of five animals each. Rats of experimental group were orally administered the suspension of the desired compounds at a dose of 100 mg/kg body weight prepared in 1.0% gum acacia. Suspension of the standard drug metformin was given at 100 mg/kg dose level. Animals of control group received vehicle (1.0% gum acacia). An oral sucrose load of 10 g/kg body weight was given to rats of all group exactly post 30 min administration of the test sample/vehicle. Blood glucose level was again measured at 30, 60, 90 and 120 min post administration of sucrose. Food but not water was withheld from the cages during the course of experimentation.
22. *Streptozotocin-induced diabetic rats.* Male albino rats of Sprague–Dawley strain (body weight 140 ± 20 g) was selected for this study. Streptozotocin was dissolved in 100 mM citrate buffer, pH 4.5, and calculated amount of the fresh solution was injected to overnight fasted rats (60 mg/kg) intraperitoneally. Blood glucose was checked 48 h later by glucometer by using glucostrips and animals showing blood glucose values between 144 and 270 mg/dl were selected and divided into groups of five animals each. Rats of experimental groups were administered suspension of the desired test samples orally (made in 1.0% gum acacia) at a dose of 100 mg/kg body weight. Animals of control group were given an equal amount of 1.0% gum acacia. A sucrose load of 2.5 g/kg of body weight was given after 30 min of drug administration. After 30 min of post-sucrose load, blood glucose level was again checked at 1, 2, 3, 4, 5 and at 6 h, respectively. Comparing the AUC of experimental and control groups determined the percent antihyperglycemic activity.
23. Shafrir, E. *Diabetes Metab. Rev.* **1992**, *8*, 179.
24. Type 2 diabetes model, that is, C57BL/KsJ-db/db mice. Hyperglycemic animals were divided into four groups having five animals each. Animals of one group were regarded as control group (orally administered 1% gum acacia) and other groups were treated as experimental groups (treated with suspension of the desired test substances at a dose of 50 mg/kg body weight). The treatment was continued for 10 consecutive days. Animals were dosed daily at a fixed time (10.00–11.00 a.m.) All animals had free access to fresh water and normal diet. Blood glucose profile of each animal was measured by glucometer using glucostrips (Boehringer Mannheim). An oral glucose tolerance test (OGTT) of each individual was performed on day 10 after an overnight fast (10 h). Blood was sampled from the tail vein at time 0 min (baseline), followed by 30, 60, 90 and 120 min after an oral glucose load of 3.0 g/kg of body weight. Quantitative glucose tolerance of each animal was calculated by area under curve (AUC) method using Prism Software. Comparing the AUC of experimental and control groups determined the percentage antihyperglycemic activity.