Research Article

Template Synthesis and Pharmacological Properties of Oxovanadium (IV) Complexes of Macrocyclic Schiff Bases

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ABSTRACT

Oxovanadium (IV) complexes of macrocyclic schiff bases have been prepared by template synthesis. The composition and structure of complexes were confirmed by melting point, molecular weight, molar conductance, magnetic moment, elemental analysis, and UV, IR and 1H-NMR spectral studies. The complexes were paramagnetic in nature as was evident from the magnetic moment measurements, which were found in the range of 1.70-1.76BM. Monomeric nature was confirmed by molecular weight determination. Some pharmacological activities like anti-inflammatory, Antipyretic, analgesic, Blood Glucose Tolerance Test and anti-diabetic activities of all the synthesized oxovanadium (IV) complexes were determined. The acute toxicity test revealed that the complexes were not toxic to the system up to 2000mg/kg dose of body weight. All the data were compared with standard drugs and analyzed as mean ± SEM.

Keywords: Oxovanadium(IV) Complexes, Anti-diabetic, Analgesic, Anti-inflammatory, Antipyretic.

INTRODUCTION

In the last few years, reports were published describing many pharmacological effects of vanadium complexes.[1-3] Many scientists have extensively reviewed the anti-diabetic effects of vanadium salts, showing that administration of vanadium salts improves glucose tolerance, lowers blood glucose levels, and corrects the metabolic deficiencies in several species of diabetic animals and human patients Vanadyl (IV) complexes, such as vanadyl cysteine methyl ester, vanadyl maloante, vanadyl tartarate, and vanadyl salicylaldehyde, normalized diabetic state in alloxan induced diabetic rats similarly to vanadate Though vanadium possesses a range of diabetes-corrective actions, its toxicity is of more concern in rodents and humans[4-7]. In most studies of the insulin-like effects of vanadium in diabetic rats and mice, oral administration of vanadium was considered to be toxic and the toxicity increased with increasing concentration of vanadium.[8] The use of various chelating agents and ligands to reduce vanadium toxicity and improve insulin potency are the major recent goals of vanadium research.[9]

Take into account these facts; we decided to investigate the effect of oxovanadium (IV) Complexes with macrocyclic Schiff bases to reduce its toxicity was formulated. In this work we synthesized some oxovanadium (IV) Complexes, characterized them and evaluated for anti-inflammatory, antipyretic, analgesic, glucose tolerance test and anti-diabetic activities.

MATERIALS AND METHODS

All chemicals and solvents were analytical reagent grade and used as received. Melting points were measured by open capillaries using Sunsim electric melting point apparatus and are uncorrected. Molecular weights were determined by Rast Camphor method. The molar conductivities of 10^{-3}M solution of the complexes in DMF were measured on Equiptronics model no.EQ-660A. Carbon, Hydrogen, Nitrogen and Metal analyses were determined on a Vario EL III Elementar Carlo Erba 1108 at CDRI, Lucknow. The IR spectra (as KBr pellets) were recorded (4600-400 cm-1) on a Perkin-Elmer 681 spectrophotometer, 1H-NMR spectra were obtained on Bruker Advance 400 MHz FT NMR spectrometer in MeOD using TMS as internal standard at CDRI, Lucknow Electronic spectra on digital spectrophotometer.. Magnetic susceptibility values were measured at room temperature by the Gouy's method using mercury tetrathiocyanatocobaltate(II) as the magnetic standard.

Preparation of PDAVO(IV) and DDMAVO(IV) Complexes

A mixture of aqueous solution of vanadium sulphate (1.26g, 1M), ethanolic solution of Acetyl acetone (0.51mL, 1M) and ethanolic solution of Phenylene diamine (0.54g, 1M) in 1:1:1 ratio were stirred then refluxed for 6-8 hours to produce PDAVO(IV) complex. To produce DDMAVO(IV) complex, the mixture of vanadium sulphate (1.26g, 1M), ethanolic solution of Acetyl acetone (0.51mL, 1M) and ethanolic solution of 4, 4-Diamino diphenyl methane(0.99g, 1M) (1:1:1) were stirred and refluxed.[10] On cooling of both solutions, colored precipitates were filtered and recrystallized with ethanol and dried in vacuum (Yield 68-70%). Proposed structures are shown in figure 1 & 2.

Preparation of PDBVO(IV) and DMBVO(IV) Complexes

The aqueous solution of vanadium sulphate (1.26g, 1M), ethanolic solution of Benzil(1.05g, 1M) and the ethanolic solution of Phenylene diamine(0.54g, 1M) were mixed in

1:1:1 ratio, stirred and refluxed for 6-8 hours to produce PDBVO(IV) complex. To produce DMBVO(IV) complex, a mixture of aqueous solution of vanadium sulphate (1.26g, 1M), ethanolic solution of Benzil(1.05g, 1M) and the ethanolic solution 4, 4-Diamino diphenyl methane(0.99g, 1M) (1:1:1) were stirred and refluxed for 6-8 hours. Both solutions were cooled, colored precipitates were then filtered and recrystallised with ethanol and dried in vacuum (Yield 67-68%). Proposed structures are shown in figure 3 & 4.

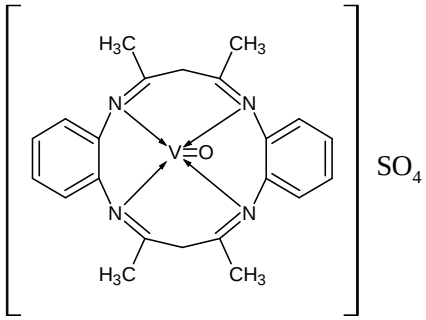

Figure 1: Proposed structure of PDAVO(IV)

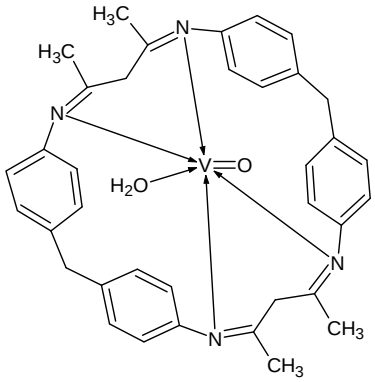

Figure 2: Proposed structure of DDMAVO(IV)

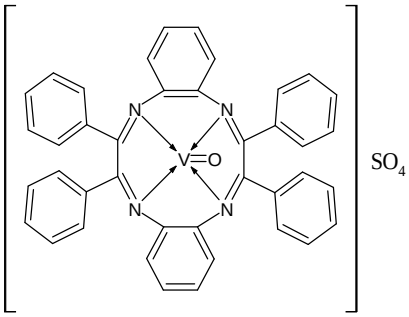

Figure 3: Proposed structure of PDBVO(IV)

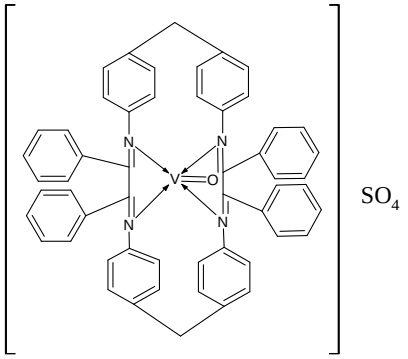

Figure 4: Proposed structure of DMBVO(IV)

Pharmacological activities

All the synthesized oxovanadium(IV) complexes were assessed for their pharmacological activities.

Selection of animals

Wistar rats of either sex weighing 100-150 g were adapted for the experiments. The animals were maintained under standard environmental conditions, were fed with a standard diet and water *ad libitum*. The animals were fasted for overnight before experimentation but allowed free access to water. The experiments were designed and conducted in accordance with the ethical norms approved by Ministry of Social Justices and Empowerment, Government of India, and Institutional Animal Ethics Committee guidelines.

Acute toxicity studies

The study for acute toxicity test was carried out as per the guidelines set by OECD. The fasted rats were divided in four groups of five animals each. All the groups were administrated with oxovanadium(IV) complexes in doses of 5, 50, 300 and 2000 mg/kg of body weight. No adverse effects or mortality were detected in the rats up to 2000mg/kg, during the 24h observation period.[11] Based on the results obtained from this study, the dose for all the pharmacological activities was fixed to be 300mg/kg of body weight (b.w.).

Anti-inflammatory activity

In this study anti-inflammatory activity was determined in overnight fasted rats by injecting carrageenan (1% w/v suspension in 0.9% saline) in the right hand paw sub plantar region using six animals in each group. The test groups of rats were given orally 300 mg/kg of body weight of oxovanadium(IV) complexes half an hour before the carrageenan injection. The control group was given the 0.5 mL of saline solution. Another group of rats was treated with standard drug diclofenac sodium (20 mg/kg b.w) orally half an hour before carrageenan injection. Increase in linear paw circumference was taken as a measure of oedema at 1, 2, 3 and 4 hour after the carrageenan injection. [12] The percent inhibition of inflammation was also calculated.

Antipyretic Activities

The antipyretic activity was evaluated using Brewer's yeast-induced pyrexia in overnight fasted rats. Fever was induced by injecting 10 mL/kg (subcutaneous) of 20 % aqueous suspension of Brewer's yeast in normal saline. Rectal temperature was recorded by digital thermometer prior to experiment. After twelve hours, the rectal temperature of each rat was again measured; only rats that showed an increase in temperature of at least 0.7°C were used for experiments. Animals were divided into six groups consisting of six animals each group. First group received control, second group received standard drug Paracetamol (150 mg/kg, b.w.), rest groups received oxovanadium(IV) complexes (300mg/kg b.w.) and the

temperature was measured at 1, 2, 3, 4, and 5 hours after drug administration.[13]

Analgesic activity

The peripheral analgesic activity of oxovanadium(IV) complexes was investigated by the acetic acid induced writhing test in rats. Oxovanadium(IV) complexes were used at the dose of 300 mg/kg b.w. orally in the study. Aspirin (150 mg/kg, b.w.) was used as standard drugs for comparing analgesic effects at peripheral level. Control group received the Saline solution only. Six animals were used in each treatment group. Thirty minutes after treatment, the rats were given an intraperitoneal (i.p.) injection of 0.6% v/v acetic acid in a volume of 10ml/kg to induce the characteristic writhings. The writhing movements were observed and counted for 20 min after acetic acid administration. The response of the each complex was compared with that of control and the % inhibition were also calculated. [14]

Blood glucose tolerance test

Fasted rats were divided into five groups of six rats each. Group I served as a control, received distilled water. Group II – V received Oxovanadium (IV) complexes at a dose of 300 mg/kg of body weight. The rats of all groups were given glucose (2 g/kg body weight,) 30min after administration of the drug. Blood samples were collected from the tail vein just prior to glucose administration and at 30 and 90 minute after the glucose loading and blood glucose levels were measured immediately by glucometer. [15]

Anti-diabetic activities

Rats were made diabetic by single intraperitoneal injection of 150mg/kg body weight of alloxan monohydrate in sterile normal saline. Three days subsequent to the injections, rats with glucose level higher than 300mg/dl were separated and used for the studies. Consequently, the rats were separated into tested groups of six animals each. The first group of animals was the control group, received saline only. Second group was treated with standard drug Glibenclamide at dose of 10 mg/kg, while rest groups were given oxovanadium(IV) complexes in a dose of 300 mg/kg of body weight. For acute study, 0.2 mL of blood sample was withdrawn through the tail vein puncture technique at interval of zero, first, third and fifth hour after oral administration and blood glucose levels were measured using blood glucose test strips with glucometer. [16-17]

Statistical analysis

In all activities, the mean ± SEM were statistically calculated for each parameter using ANOVA. Statistical significance was determined by using student's t-test to study the differences amongst the means.

RESULTS AND DISCUSSION

The synthesized oxovanadium(IV) complexes were intensely coloured. The electrical conductivities of 10^{-3}M solution of the complexes measured in DMF are low, with values less than 20 ohm^{-1} cm^2 mol^{-1} indicating non electrolytical nature of the compounds. Molecular weights determined by Rast Camphor method were found in accordance with calculated value, confirming the monomeric nature of the compounds. Magnetic moment was found to be in range of 1.70-1.76BM, which fall in the expected range of paramagnetic character of the compounds. Micro analytical data are shown in table 1.

Table 1: Micro analytical data of Compounds

Compound	Yield in %	Color	MP IN °C	MW F(C)	Element analysis in % found (CALC)				MAG. Moment
					C	H	N	V	
PDAVO(IV) $C_{22}H_{24}N_4OV$	68	Brownish Black	120	(410) 412	62.95 (64.07)	6.01 (5.82)	14.18 (13.59)	12.62 (12.64)	1.70BM
DDMAVO(IV) $C_{36}H_{36}N_4OV$	70	Greenish Black	Above 200	(590) 592	71.68 (72.97)	7.19 (6.08)	9.21 (9.45)	9.01 (8.78)	1.75BM
PDBVO(IV) $C_{40}H_{28}N_4OV$	67	Greenish Black	130	630 (632)	75.6 (75.9)	4.7 (4.4)	8.6 (8.8)	8.1 (8.2)	1.76BM
DMBVO(IV) $C_{30}H_{22}N_6S_2OV$	68	Greenish Black	190	794 (812)	80.3 (79.9)	4.1 (4.9)	7.3 (6.9)	6.7 (6.2)	1.71BM

All the spectral data was consistent with the assigned structure of the compounds. Electronic spectra of all oxovanadium(IV) complexes showed two bands first between 245-300nm and another between 280- 325nm attributable to π- π* and n-π* transition respectively. From the IR spectra of the oxovanadium(IV) complexes the band in region 1550-1610cm-1, validated the occurrence of C=N group. This band gets shifted to lower frequency in the complexes, indicating the coordination through azomethine nitrogen. It is also found from these spectra that there are wide and strong band at 985 – 994 cm^{-1}, which are assigned to V=O stretching vibration. The complex DDMAVO(IV) exhibited the broad band between 3200-3500 cm^{-1}, together with a band between 830-840 cm–1 indicating the presence of coordinated water. The rest complexes PDAVO(IV), PDBVO(IV) and DMBVO(IV) illustrated three bands at 1130-1140, 955-960 and 600-610 cm^{-1}.these confirmed the presence of ionic sulphate

group The ^{1}HNMR spectra of complexes showed signal between δ7.22-7.99 due to aromatic ring. The proton signal for methyl group in complexes PDAVO(IV) and DDMAVO(IV) appeared between δ2.13-2.91.

From all the micro analytical and spectral data the proposed structure of all the oxovanadium(IV) complexes were confirmed with having square pyramidal geometries.

Anti-inflammatory activity

The oxovanadium(IV) complexes significantly (as compared to control) reduced carrageenan induced paw oedema in rats. The standard drug diclofenac sodium showed (97.14%) of inhibitory activity while compounds showed the same between 84.28-94.28% as shown in Table 2. Thus the inhibitory activity of different complexes was found to be close to standard drug. Also their graphical presentation is shown in figure 5.

Table 2: Anti-inflammatory action of Compounds in carrageenan induced paw oedema

Compounds	Mean ±SEM increase in paw volume and % inhibition			
	1hr	2 hr	3 hr	4 hr
Control	3.18±0.091	3.27±0.089	3.16±0.124	3.05±0.105
Standard (Diclofenac)	2.49±0.214 (67.46%)	2.52±0.191 (67.39%)	2.39±0.195 (79.01%)	2.24±0.206 (97.14%)
DMBVO(IV)	2.47±0.158 (66.26%)	2.40±0.218 (77.17%)	2.33±0.315 (82.71%)	2.23±0.114 (94.28%)
DDMAVO(IV)	2.65±0.117 (63.85%)	2.80±0.215 (51.08%)	2.62±0.312 (66.66%)	2.40±0.218 (91.42%)
PDBVO(IV)	2.51±0.101 (62.65%)	2.62±0.428* (54.34%)	2.46±0.220 (67.90%)	2.29±0.112 (87.14%)
PDAVO(IV)	2.39±0.216 (56.62%)	2.40±0.309 (59.78%)	2.28±0.213 (69.13%)	2.14±0.324* (84.28%)

*Significance level P< 0.001, *P< 0.01 (n=6)*

Table 3: Effect of Compounds on brewer's yeast induced pyrexia in rats

Compounds	Rectal temperature in °C at time (hr) and % Inhibition					
	0	1	2	3	4	5
Control	38.34±0.174	38.26±0.154	38.35±0.232	38.18±0.159	38.27±0.511	38.19±0.308
Standard (Paracetamol)	38.12±0.254	38.02±0.113 (12.24%)	37.84±0.236 (36.44%)	37.56±0.212 (55.55%)	37.43±0.139 (72.72%)	37.17±0.537 (98.90%)
PDAVO(IV)	37.42±0.156	37.57±0.233 (10.28%)	37.33±0.327 (40.18%)	37.12±0.321 (52.22%)	36.98±0.215 (70.70%)	36.73±0.129 (95.60%)
PDBVO(IV)	37.71±0.284	37.61±0.148 (8.16%)	37.49±0.127 (27.10%)	37.32±0.414 (32.22%)	37.08±0.321 (62.62%)	36.77±0.236 (93.4%)
DMBVO(IV)	37.90±0.198	37.73±0.342 (7.14%)	37.59±0.120 (28.03%)	37.27±0.406 (50.0%)	37.06±0.213 (75.75%)	36.89±0.421 (92.3%)
DDMAVO(IV)	38.44±0.365	38.14±0.235 (4.08%)	37.86±0.174 (38.31%)	37.61±0.271 (54.49%)	37.52±0.416 (67.67%)	37.31±0.422 (87.91%)

Significance level P< 0.001 (n=6)

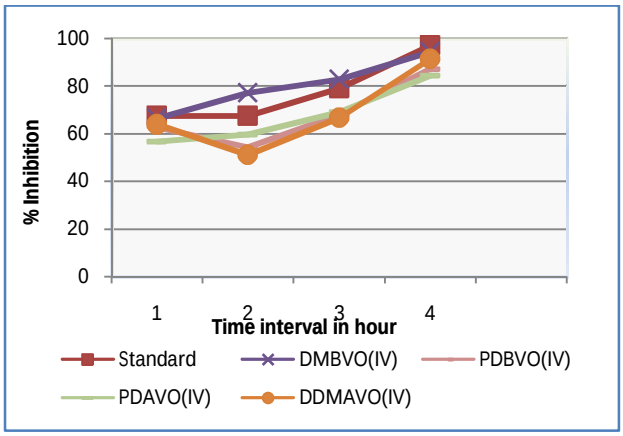

Figure 5: Graphical representation of Anti-inflammatory action of Compounds

Antipyretic Activities

Rectal temperatures of rats before yeast injection ranged from 36.71 to 37.28°C. Subcutaneous injection of 20% yeast increased the rectal temperature to between 37.42 to 38.44°C after 18 hours. Administration of oxovanadium(IV) complexes reduced the elevated temperature up to 4.08-10.28% within first hour. Temperature was reduced to between 87.91-95.60% a fifth hour, which was up to 98.90% in case of standard drug paracetamol as shown in table 3 and their curves in Figure 6.

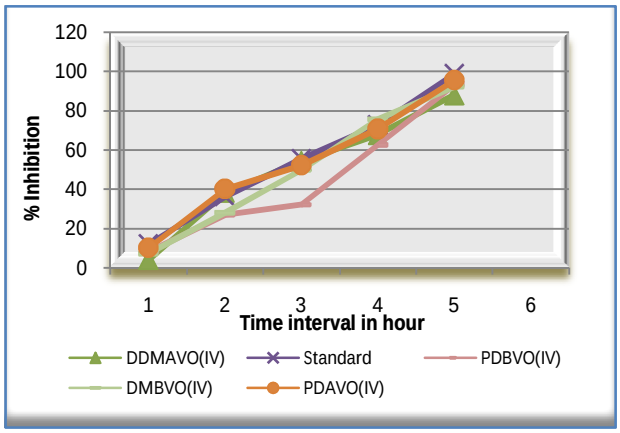

Figure 6: Graphical representation of Antipyretic activities of compounds

Analgesic activity

The effects of oxovanadium(IV) complexes on acetic-acid induced writhes in rats have been shown in Table 4. All the compounds produced a significant decrease in no. of writhes in comparison with the control group. Aspirin showed around 75% of inhibition while compounds found to be in range of 43.75-60.41% of inhibition.

Table 4: Analgesic effects of Compounds on acetic-acid induced writhings

Compounds	No. of writhings mean ± SEM	% Inhibition
Control	48±0.549	-
Standard(Aspirin)	12±0.493	75
PDBVO(IV)	19±0.227	60.41
DMBVO(IV)	22±0.514	54.16
DDMAVO(IV)	25±0.618	47.91
PDAVO(IV)	27±0.382	43.75

Significance level P< 0.001 (n=6)

Blood glucose tolerance test

The effects of blood glucose tolerance test have been shown in table 5 and their curves in figure 7. The blood glucose levels were reduced to near normal range considerably within 90 minutes of the drug administration.

Table 5: Effects of Compounds on glucose tolerance in rats

Compounds	Blood glucose (mg/dl)		
	Fasting	30 min	90min
Glucose; 2gms	90± 0.152	146± 0.215	152± 0.139
PDBVO(IV)	88± 0.129	141± 0.307	95± 0.251
DMBVO(IV)	89± 0.165	127± 0.298	97± 0.190
PDAVO(IV)	91± 0.247	126± 0.383	98± 0.288
DDMAVO(IV)	90± 0.230	130± 0.116	100± 0.235

Significance level P< 0.001 (n=6)

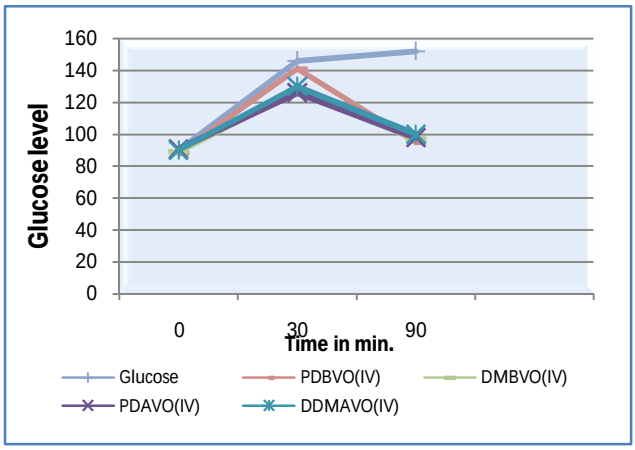

Figure 7: Graphical representation of Effects of Compounds on glucose tolerance test

Anti-diabetic activities

The results of effect of oxovanadium(IV) complexes in alloxan induced diabetic rats, which are expressed as change in blood glucose level at different time interval are shown in table 6, The blood glucose levels were reduced considerably within 60 minutes of the drug administration. At fifth hour % inhibition was found to be in range 39.03-37.28%.

Maximum effect was observed for DDMAVO(IV) complex. Treatment of the diabetic rats with Glibenclamide produced (40.35%) fall of blood glucose after 5h treatment. Graphical representation is also shown in figure 8.

Table 6: Effect of Compounds on blood glucose level (mg/dl) in alloxan-induced diabetic rats

Compounds	Blood glucose level in mg/dl (Mean ± SEM) (% Inhibition)			
	0 hour	1 hour	3 hour	5 hour
Control	320±0.045	322±0.051	321±0.050	318±0.059
Standard (Glibenclamide)	313±0.061	280±0.066 (17.67%)	246±0.0432 (32.03%)	225±0.049 (40.35%)
DDMAVO(IV)	312±0.054	282±0.041 (16.81%)	250±0.056 (30.30%)	228±0.035 (39.03%)
PDBVO(IV)	315±0.038	283±0.058 (15.94%)	252±0.042 (29.00%)	229±0.065 (38.15%)
PDAVO(IV)	314±0.067	285±0.052 (15.51%)	255±0.059 (28.13%)	231±0.055 (37.71%)
DMBVO(IV)	315±0.053	286±0.062 (15.08%)	254±0.046 (28.57%)	232±0.039 (37.28%)

Significance level P< 0.001 (n=6)

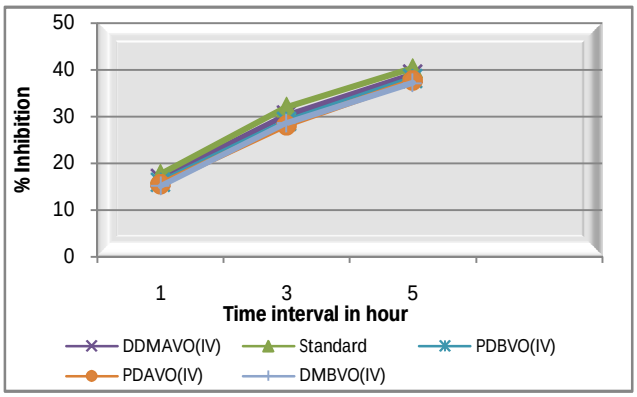

Figure 8: Graphical representation of Anti-diabetic action of compounds

CONCLUSION

In conclusion, we have demonstrated that all the tested Oxovanadium(IV) complexes has anti-inflammatory, antipyretic, analgesic and anti-diabetic activities which are comparable to respective standard drugs. Hence may be potentially useful in the management of these conditions in clinical areas.

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