BIOGENIX RESEARCH CENTER
CENTER FOR MOLECULAR BIOLOGY AND APPLIED SCIENCE
KRRA 31 - KESHAVADEV ROAD - POOJAPPURA - THIRUVANANTHAPURAM
Phone : 0471 - 3229192
E-mail : info@biogenixresearchcenter.com
Web : www.biogenixresearchcenter.com
REG NO : 2427/14

IN VITRO ANTI-INFLAMMATORY ACTION- TEST REPORT

- Sample A: 1,8 Cineol
- Sample B: Recovereez (Cardamom extract)
- Sample C: Memoreez (Cardamom extract and Virgin coconut oil)
- ANTI-INFLAMMATORY ACTION by ELISA assay in RAW 264.7 (monocyte/macrophages) cells showed significant potential to decrease prominent inflammatory markers such as IL-6, IL1-β, TNF-α, TGF-β, COX, LOX, MPO, iNOS and CELLULAR NITRATE LEVELS confirming the anti-inflammatory nature of 1,8-Cineol, Recovereez and Memoreez capsules.
- All the samples in the tested concentrations as given in the detailed report which is attached below showed dose dependent anti-inflammatory action in the selected assays.

Dr. NITHIN VIJAYAKUMAR
AUTHORIZED SIGNATORY

BIOGENIX RESEARCH CENTRE BGRC Reg. No. 594/07 THIRUVANANTHAPURAM

Dr. RAJESH RAMACHANDRAN
RESEARCH HEAD AND DIRECTOR

NB: The experiments are carried out in *invitro* cultured cells and Intended only for research purposes not for diagnostic uses. All experiments are carried out only in triplicates

ANTI- INFLAMMATORY ASSAYS

RAW 264.7 cellswas initially procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained Dulbecco's modified Eagles medium, DMEM (Sigma aldrich, USA).

The cell line was cultured in 25 cm^2 tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate (Merck, Germany) and antibiotic solution containing: Penicillin (100U/ml), Streptomycin (100µg/ml), and Amphoteracin B (2.5µg/ml). Cultured cell lines were kept at 37ºC in a humidified 5% CO_2 incubator (NBS Eppendorf, Germany).

The cells were grown to 60% confluency followed by activation with 1 µL lipopolysaccharide (LPS: 1µg/mL). LPS stimulated RAW cells were exposed with different concentration (25,50, 100 µg/mL) of sample solution and Diclofenac sodium, a standard anti-inflammatory drug in varying concentration corresponding to the sample was added and incubated for 24 hours. After incubation the anti-inflammatory assays were performed using the cell lysate.

Cyclooxygenase (COX) activity

The COX activity was assayed by the method of Walker and Gierse. 100µl cell lysate was incubated withTris-HCl buffer (pH 8), glutathione 5 mM/L, and hemoglobin 5 mM/L for 1 minute at 25°C. The reaction was initiated by the addition ofarachidonic acid 200 mM/L and terminated after 20 minutes incubation at 37°C, by the addition 200µL of 10% trichloroacetic acid in 1 N hydrochloric acid. After the centrifugal separation and the addition of 200µL of 1% thiobarbiturate, the tubes were boiled for 20 minutes. After cooling, the tubes were centrifuged for three minutes. COX activity was determined by reading absorbance at 632 nm.

Calculation

Percentage inhibition of the enzyme was calculated as,

% inhibition = ((Absorbance of control-Absorbance of test)/Absorbance of control) × 100

Sample Concentration (µg/ml)	OD I	OD II	OD III	Average of OD	Percentage inhibition

LPS	0.0975	0.0984	0.097	0.0976	0.0976
A					
6.25	0.0814	0.0802	0.0847	0.0821	15.88
12.5	0.0622	0.0683	0.0657	0.0654	32.99
25	0.0406	0.039	0.0398	0.0398	59.22
B					
6.25	0.0784	0.079	0.0785	0.0786	19.43
12.5	0.0546	0.0581	0.0598	0.0575	41.08
25	0.0364	0.0341	0.0329	0.0344	64.68
C					
6.25	0.0714	0.0723	0.0731	0.0722	25.95
12.5	0.0494	0.0491	0.0476	0.0487	50.10
25	0.0326	0.0354	0.0329	0.0336	65.53

Sample Concentration (µg/ml)	**Percentage inhibition 1**	**Percentage inhibition 2**	**Percentage inhibition 3**	**Average**	**Stdev**	**Std error**
SAMPLE A						
LPS	0	0	0	0	0	0
6.25	16.51282	18.49593	12.68041	15.89639	2.95636	0.985453
12.5	36.20513	30.58943	32.26804	33.02087	2.882546	0.960849
25	58.35897	60.36585	58.96907	59.2313	1.028817	0.342939
SAMPLE B						
LPS	0	0	0	0	0	0
6.25	19.58974	19.71545	19.07216	19.45912	0.340955	0.113652

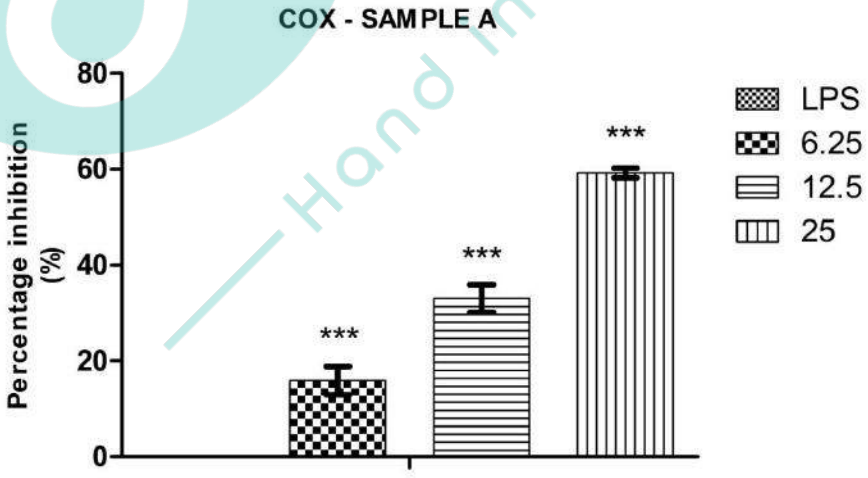
12.5	44	40.95528	38.35052	41.10193	2.827596	0.942532
25	62.66667	65.34553	66.08247	64.69822	1.797551	0.599184
SAMPLE C						
LPS	0	0	0	0	0	0
6.25	26.76923	26.52439	24.63918	25.9776	1.165556	0.388519
12.5	49.33333	50.10163	50.92784	50.12093	0.797426	0.265809
25	66.5641	64.02439	66.08247	65.55699	1.348939	0.449646

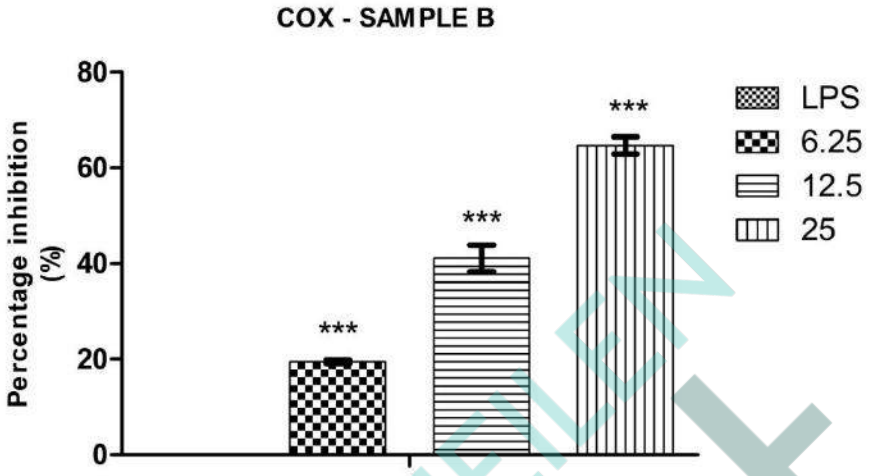
Table Analyzed	**Sample A**				
One-way analysis of variance					
P value	< 0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	4				
F	425.8				
R squared	0.9938				
ANOVA Table	SS	df	MS		
Treatment (between columns)	5782	3	1927		
Residual (within columns)	36.22	8	4.527		
Total	5818	11			
Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
LPS vs 6.25	-15.90	9.150	Yes	***	-20.90 to -10.89
LPS vs 12.5	-33.02	19.01	Yes	***	-38.02 to -28.02
LPS vs 25	-59.23	34.10	Yes	***	-64.23 to -54.23

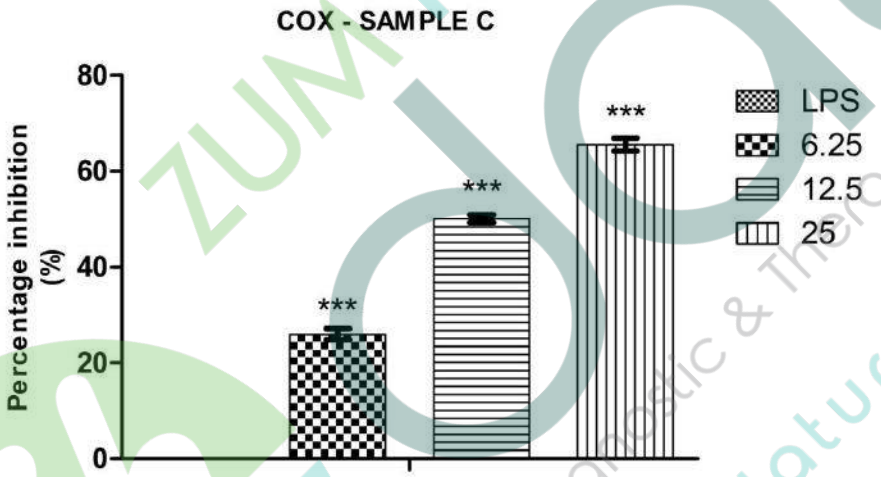
Table Analyzed	**SAMPLE B**				
One-way analysis of variance					
P value	< 0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	4				
F	822.2				
R squared	0.9968				
ANOVA Table	SS	df	MS		
Treatment (between columns)	6994	3	2331		

Residual (within columns)	22.69	8	2.836		
Total	7017	11			
Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
LPS vs 6.25	-19.46	14.15	Yes	***	-23.42 to -15.50
LPS vs 12.5	-41.10	29.89	Yes	***	-45.06 to -37.14
LPS vs 25	-64.70	47.06	Yes	***	-68.66 to -60.74

Table Analyzed	**SAMPLE C**				
One-way analysis of variance					
P value	< 0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	4				
F	2588				
R squared	0.9990				
ANOVA Table	SS	df	MS		
Treatment (between columns)	7404	3	2468		
Residual (within columns)	7.628	8	0.9535		
Total	7412	11			
Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
LPS vs 6.25	-25.98	32.58	Yes	***	-28.27 to -23.68
LPS vs 12.5	-50.12	62.86	Yes	***	-52.42 to -47.82
LPS vs 25	-65.56	82.22	Yes	***	-67.85 to -63.26







Lipoxygenase (LOX) activity

The determination of LOX activity was done as per Axelrod *et al*. Briefly, the reaction mixture (2 mL final volume) contained Tris-HCl buffer (pH 7.4), 50 µL of cell lysate, and sodium linoleate (200 µL). The LOX activity was monitored as an increase of absorbance at 234 nm (Shimadzu), which reflects the formation of 5-hydroxyeicosatetraenoic acid.

Calculation

Percentage inhibition of the enzyme was calculated as,

% inhibition = ((Absorbance of control-Absorbance of test)/Absorbance of control) × 100

Sample Concentration (µg/ml)	OD I	OD II	OD III	Average OD	Percentage inhibition
LPS	0.3262	0.3194	0.3116	0.319067	0
A					
6.25	0.2802	0.2842	0.2911	0.285167	10.60
12.5	0.1571	0.1547	0.1492	0.153667	51.82
25	0.1018	0.1062	0.104	0.104	67.39
B					
6.25	0.2894	0.279	0.2766	0.2816	11.70
12.5	0.1482	0.1501	0.1422	0.1468	53.97
25	0.0872	0.0857	0.0889	0.0872	72.64
C					
6.25	0.2767	0.2754	0.2667	0.2729	14.44
12.5	0.1328	0.1317	0.1348	0.1331	58.27
25	0.0772	0.0754	0.0781	0.0769	75.89

Sample Concentration (µg/ml)	Percentage inhibition 1	Percentage inhibition 2	Percentage inhibition 3	Average	Stdev	Std error
SAMPLE A						
LPS	0	0	0	0	0	0
6.25	14.10178	11.02066	6.578947	10.56713	3.781867	1.260622
12.5	51.83936	51.56544	52.1181	51.84097	0.276336	0.092112
25	68.79215	66.75016	66.62388	67.38873	1.217039	0.40568
SAMPLE B						
LPS	0	0	0	0	0	0
6.25	11.28142	12.64872	11.23235	11.72083	0.803948	0.267983
12.5	54.56775	53.00564	54.36457	53.97932	0.849332	0.283111
25	73.26793	73.16844	71.46983	72.6354	1.010638	0.336879
SAMPLE C						
LPS	0	0	0	0	0	0
6.25	15.17474	13.77583	14.4095	14.45336	0.700485	0.233495
12.5	59.28878	58.76644	56.73941	58.26488	1.346661	0.448887
25	76.33354	76.39324	74.93582	75.88753	0.82475	0.274917

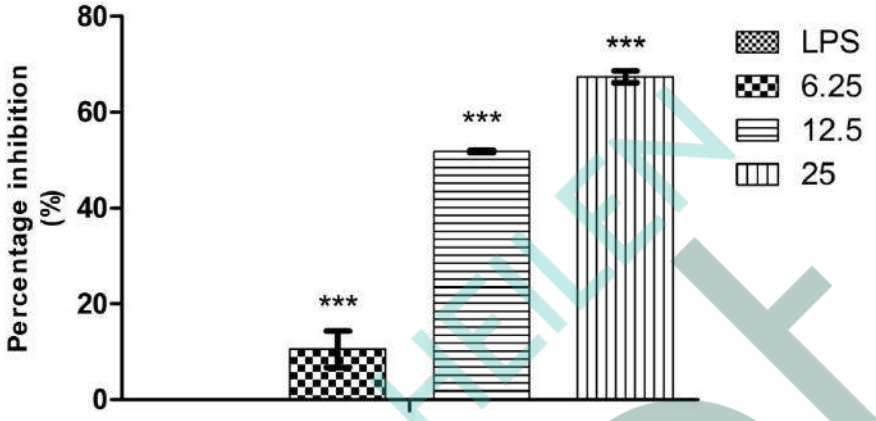
Table Analyzed	SAMPLE A				
One-way analysis of variance					
P value	< 0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	4				
F	789.0				
R squared	0.9966				
ANOVA Table	SS	df	MS		
Treatment (between columns)	9386	3	3129		
Residual (within columns)	31.72	8	3.965		
Total	9417	11			
Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Table Analyzed	Sample B				

LPS vs 6.25	-10.57	6.500	Yes	***	-15.25 to -5.885
LPS vs 12.5	-51.84	31.89	Yes	***	-56.52 to -47.16
LPS vs 25	-67.39	41.45	Yes	***	-72.07 to -62.71

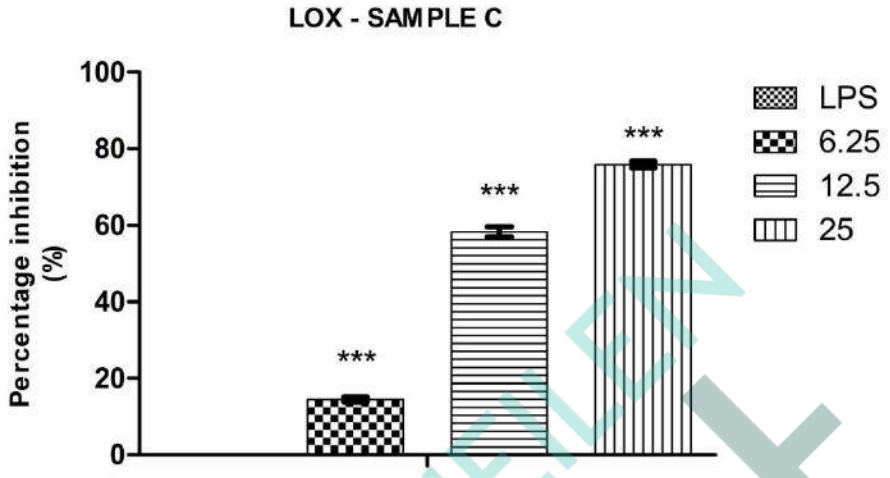
One-way analysis of variance					
P value	< 0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	4				
F	5932				
R squared	0.9996				
ANOVA Table	SS	df	MS		
Treatment (between columns)	10630	3	3543		
Residual (within columns)	4.778	8	0.5973		
Total	10630	11			
Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
LPS vs 6.25	-11.72	18.57	Yes	***	-13.54 to -9.904
LPS vs 12.5	-53.98	85.54	Yes	***	-55.80 to -52.16
LPS vs 25	-72.64	115.1	Yes	***	-74.45 to -70.82

Table Analyzed	Sample C				
One-way analysis of variance					
P value	< 0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	4				
F	5149				
R squared	0.9995				
ANOVA Table	SS	df	MS		
Treatment (between columns)	11530	3	3842		
Residual (within columns)	5.969	8	0.7461		
Total	11530	11			
Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
LPS vs 6.25	-14.45	20.49	Yes	***	-16.48 to -12.42
LPS vs 12.5	-58.26	82.61	Yes	***	-60.30 to -56.23
LPS vs 25	-75.89	107.6	Yes	***	-77.92 to -73.86

LOX - SAMPLE A



LOX - SAMPLE B



Myeloperoxidase (MPO) activity

Cell lysate was homogenized in a solution containing 50 mM potassium phosphate buffer and 0.57% hexadecyltrimethyl ammonium bromide (HTAB) the samples were centrifuged at 2000 g for 30 minutes at 4°C, and supernatant was assayed for MPO activity. MPO in the sample was activated by the addition of 50 mM phosphate buffer (pH 6) containing 1.67 mg/mL guaiacol and 0.0005% H 2 O. The change in absorbance at 460 nm was measured. MPO activity was presented as units per mL of cell lysate. One unit of MPO activity was defined as that degrading 1 µM of peroxide per minute at 25°C.

U = (ΔOD · 4 · Vt · dilution factor) / (L · £470 ·Δt · Vs)

ΔOD = density change

Vt = total volume (mL) (1.1 mL)

L=light path (1 cm)

£470 = extinction coefficient for tetraguaiacol (26.6 mM-1·cm-1,)

Sample Concentration (µg/ml)	OD I	OD II	OD III	Average OD	Enzyme Activity (U/ml)
LPS	0.0788	0.0745	0.0796	0.077633	0.5302
A					
6.25	0.0712	0.0709	0.0699	0.070667	0.4826
12.5	0.0625	0.0635	0.064	0.063333	0.4325
25	0.0574	0.0562	0.0583	0.0573	0.3913
B					
6.25	0.069	0.0684	0.0697	0.069033	0.4714
12.5	0.0618	0.0603	0.0612	0.0611	0.4173
25	0.054	0.0521	0.05	0.052033	0.3553
C					
6.25	0.0654	0.066	0.0642	0.0652	0.4453
12.5	0.0576	0.0582	0.0571	0.057633	0.3936
25	0.0498	0.051	0.0499	0.050233	0.3430

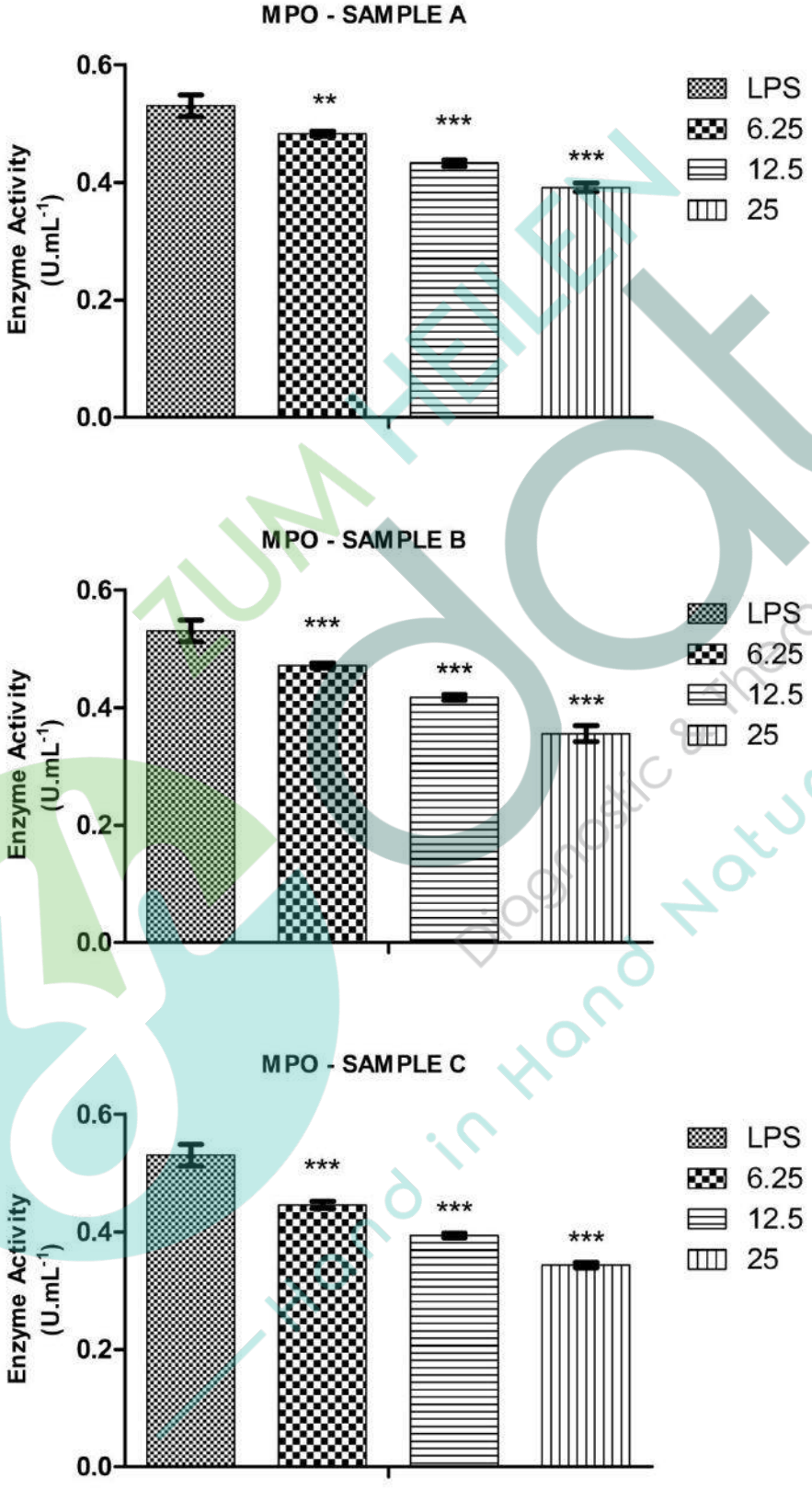
Sample Concentration (µg/ml)	Enzyme activity (U/mL) 1	Enzyme activity (U/mL) 2	Enzyme activity (U/mL) 3	Average	Stdev	Std Error
SAMPLE A						
LPS	0.538204	0.508835	0.543668	0.530236	0.018734	0.006245
6.25	0.486296	0.484247	0.477417	0.482653	0.004649	0.00155
12.5	0.426875	0.433705	0.43712	0.432567	0.005216	0.001739
25	0.392042	0.383846	0.398189	0.391359	0.007196	0.002399
SAMPLE B						
LPS	0.538204	0.508835	0.543668	0.530236	0.018734	0.006245
6.25	0.47127	0.467172	0.476051	0.471498	0.004444	0.001481
12.5	0.422094	0.411849	0.417996	0.417313	0.005157	0.001719
25	0.36882	0.355843	0.3415	0.355388	0.013666	0.004555
SAMPLE C						
LPS	0.538204	0.508835	0.543668	0.530236	0.018734	0.006245
6.25	0.446682	0.45078	0.438486	0.445316	0.00626	0.002087
12.5	0.393408	0.397506	0.389993	0.393636	0.003762	0.001254
25	0.340134	0.34833	0.340817	0.343094	0.004548	0.001516

Table Analyzed	Sample A				
One-way analysis of variance					
P value	< 0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	4				
F	96.62				
R squared	0.9731				
ANOVA Table	SS	df	MS		
Treatment (between columns)	0.03272	3	0.01091		
Residual (within columns)	0.0009031	8	0.0001129		
Total	0.03363	11			
Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff

LPS vs 6.25	0.04758	5.485	Yes	**	0.02260 to 0.07256
LPS vs 12.5	0.09767	11.26	Yes	***	0.07269 to 0.1227
LPS vs 25	0.1389	16.01	Yes	***	0.1139 to 0.1639

Table Analyzed	Sample B				
One-way analysis of variance					
P value	< 0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	4				
F	114.8				
R squared	0.9773				
ANOVA Table	SS	df	MS		
Treatment (between columns)	0.05027	3	0.01676		
Residual (within columns)	0.001168	8	0.0001460		
Total	0.05144	11			
Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
LPS vs 6.25	0.05874	5.954	Yes	***	0.03033 to 0.08715
LPS vs 12.5	0.1129	11.45	Yes	***	0.08451 to 0.1413
LPS vs 25	0.1748	17.72	Yes	***	0.1464 to 0.2033

Table Analyzed	Sample C				
One-way analysis of variance					
P value	< 0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	4				
F	180.2				
R squared	0.9854				
ANOVA Table	SS	df	MS		
Treatment (between columns)	0.05743	3	0.01914		
Residual (within columns)	0.0008499	8	0.0001062		
Total	0.05828	11			
Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
LPS vs 6.25	0.08492	10.09	Yes	***	0.06068 to 0.1092
LPS vs 12.5	0.1366	16.23	Yes	***	0.1124 to 0.1608
LPS vs 25	0.1871	22.24	Yes	***	0.1629 to 0.2114


MPO - SAMPLE A
Enzyme Activity (U.mL-1)
0.6
0.4
0.2
0.0
**
***
***
LPS
6.25
12.5
25
MPO - SAMPLE B
Enzyme Activity (U.mL-1)
0.6
0.4
0.2
0.0
***
***
***
LPS
6.25
12.5
25
MPO - SAMPLE C
Enzyme Activity (U.mL-1)
0.6
0.4
0.2
0.0
***
***
***
LPS
6.25
12.5
25

Inducible Nitric Oxide Synthase

Nitric oxide synthase was determined by the method described by Salter et.al 1997.

Cell lysate was homogenized in 2ml of HEPES buffer. The assay system contained substrate 0.1ml -2µmol/L L-Arginine, 0.1ml- 4µmol/L manganese chloride, 0.1ml-10mmol/L 30µg dithiothreitol (DTT), 0.1ml- 1mmol/L NADPH, 0.1ml- 4µmol/L tetrahydropterin, 0.1 ml 10µmol/L oxygenated haemoglobin and 0.1ml enzyme (sample). Increase in absorbance was recorded at 401nm.

Calculation

Percentage inhibition of the enzyme was calculated as,

% inhibition = ((Absorbance of control-Absorbance of test)/Absorbance of control) × 100

Sample Concentration (µg/ml)	OD I	OD II	OD III	Average OD	Percentage of Inhibition
LPS	0.0641	0.0689	0.0664	0.066467	0
A					
6.25	0.0526	0.0547	0.0542	0.053833	19.16917
12.5	0.0321	0.0311	0.0301	0.0311	53.3033
25	0.023	0.0219	0.0242	0.023033	65.41542
B					
6.25	0.0499	0.0501	0.0521	0.0507	23.87387
12.5	0.0298	0.0297	0.0289	0.029467	55.75576
25	0.0195	0.0198	0.0206	0.019967	70.02002
C					
6.25	0.0522	0.0517	0.0532	0.052367	21.37137
12.5	0.0281	0.0275	0.0279	0.027833	58.20821

25	0.0189	0.0188	0.0192	0.018967	71.52152

Sample Concentration (µg/ml)	Percentage inhibition 1	Percentage inhibition 2	Percentage inhibition 3	Average	Stdev	Std error
SAMPLE A						
LPS	0	0	0	0	0	0
6.25	17.94072	20.60958	18.37349	18.9746	1.432375	0.477458
12.5	49.922	54.86212	54.66867	53.15093	2.798011	0.93267
25	64.11856	68.2148	63.55422	65.29586	2.543578	0.847859
SAMPLE B						
LPS	0	0	0	0	0	0
6.25	22.15289	27.28592	21.53614	23.65832	3.156696	1.052232
12.5	53.51014	56.89405	56.4759	55.6267	1.844877	0.614959
25	69.57878	71.2627	68.9759	69.93913	1.18522	0.395073
SAMPLE C						
LPS	0	0	0	0	0	0
6.25	18.56474	24.96372	19.87952	21.13599	3.379461	1.126487
12.5	56.16225	60.08708	57.98193	58.07709	1.964148	0.654716
25	70.51482	72.71408	71.08434	71.43775	1.141427	0.380476

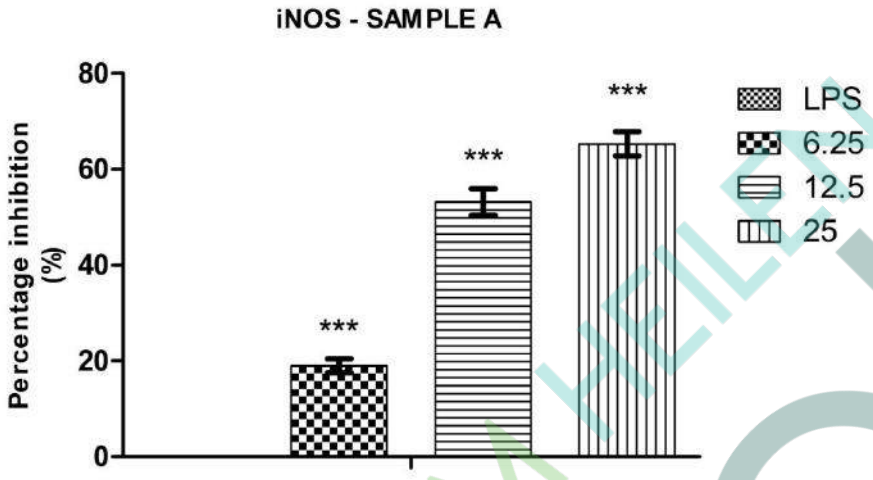
Table Analyzed	Sample A				
One-way analysis of variance					
P value	< 0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	4				
F	667.3				
R squared	0.9960				
ANOVA Table	SS	df	MS		
Treatment (between columns)	8182	3	2727		

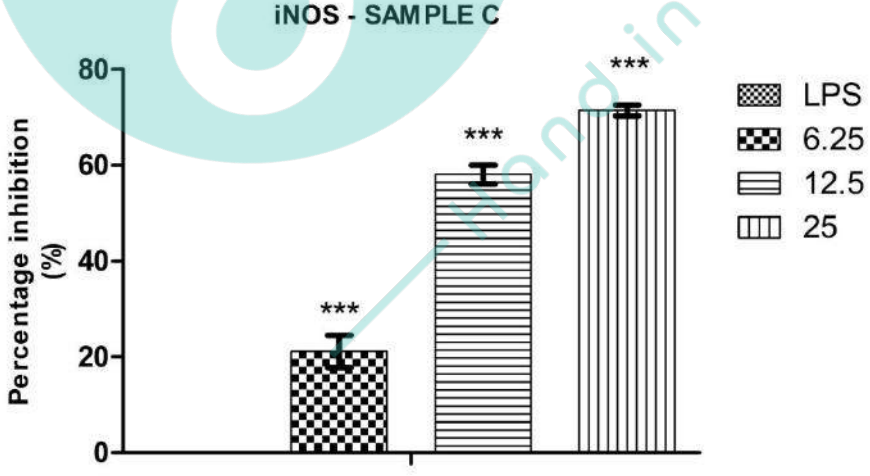
Residual (within columns)	32.70	8	4.088		
Total	8215	11			
Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
LPS vs 6.25	-18.97	11.49	Yes	***	-23.73 to -14.22
LPS vs 12.5	-53.15	32.20	Yes	***	-57.90 to -48.40
LPS vs 25	-65.30	39.55	Yes	***	-70.05 to -60.54

Table Analyzed	Sample B				
One-way analysis of variance					
P value	< 0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	4				
F	806.5				
R squared	0.9967				
ANOVA Table	SS	df	MS		
Treatment (between columns)	8936	3	2979		
Residual (within columns)	29.55	8	3.693		
Total	8965	11			
Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
LPS vs 6.25	-23.66	15.08	Yes	***	-28.18 to -19.14
LPS vs 12.5	-55.63	35.45	Yes	***	-60.15 to -51.11
LPS vs 25	-69.94	44.57	Yes	***	-74.46 to -65.42

Table Analyzed	Sample C				
One-way analysis of variance					
P value	< 0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	4				
F	783.8				
R squared	0.9966				
ANOVA Table	SS	df	MS		
Treatment (between columns)	9747	3	3249		
Residual (within columns)	33.16	8	4.145		
Total	9780	11			
Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
LPS vs 6.25	-21.14	12.71	Yes	***	-25.92 to -16.35
LPS vs 12.5	-58.08	34.94	Yes	***	-62.86 to -53.29

LPS vs 25	-71.44	42.97	Yes	***	-76.22 to -66.65





Estimation of Cellular Nitrite Levels

The level of nitrite level was estimated by the method of Lepoivre et al. (Lepoivre et. al. 1990) To 0.5 mL of cell lysate, 0.1 mL of 3% sulphosalicylic acid was added and vortexed well for 30 minutes. The samples were then centrifuged at 5,000 rpm for 15 minutes. The protein-free supernatant was used for the estimation of nitrite levels. To 200 µL of the supernatant, 30 µL of 10% NaOH was added, followed by 300 µL of Tris-HCl buffer and mixed well. To this, 530 µL of Griess reagent(1% sulphanilamide,2% phosphoric acid and 0.1% N-1-naphthyl ethylene diaminedihydrochloride) was added and incubated in the dark for 10–15 minutes, and the absorbance was read at 540 nm against a Griess reagent blank. Sodium nitrite solution was used as the standard. The amount of nitrite present in the samples was estimated from the standard curves obtained.

Sample Concentration (µg/ml)	OD I	OD II	OD III	Average OD	Concentration of Nitrite (µg)
LPS	0.0936	0.0986	0.0944	0.095533	472.89
A					
6.25	0.0789	0.0766	0.0736	0.076367	378.015
12.5	0.0598	0.0589	0.0547	0.0578	286.11
25	0.0407	0.0416	0.0447	0.042333	209.55
B					
6.25	0.0694	0.0688	0.0699	0.069367	343.365
12.5	0.0587	0.0564	0.0579	0.057667	285.45
25	0.04	0.0394	0.0375	0.038967	192.885
C					
6.25	0.0677	0.0661	0.0659	0.066567	329.505

12.5	0.0511	0.0497	0.05	0.050267	248.82
25	0.0361	0.0342	0.0365	0.0356	176.22

Sample Concentration (µg/ml)	Concentration of Nitrite (µg) 1	Concentration of Nitrite (µg) 2	Concentration of Nitrite (µg) 3	Average	Stdev	Std error
SAMPLE A						
LPS	463.32	488.07	467.28	472.89	13.29454	4.431512
6.25	390.555	379.17	364.32	378.015	13.15558	4.385194
12.5	296.01	291.555	270.765	286.11	13.47455	4.491517
25	201.465	205.92	221.265	209.55	10.38714	3.46238
SAMPLE B						
LPS	463.32	488.07	467.28	472.89	13.29454	4.431512
6.25	343.53	340.56	346.005	343.365	2.726247	0.908749
12.5	290.565	279.18	286.605	285.45	5.779712	1.926571
25	198	195.03	185.625	192.885	6.460335	2.153445
SAMPLE C						
LPS	463.32	488.07	467.28	472.89	13.29454	4.431512
6.25	335.115	327.195	326.205	329.505	4.883554	1.627851
12.5	252.945	246.015	247.5	248.82	3.648702	1.216234
25	178.695	169.29	180.675	176.22	6.082662	2.027554

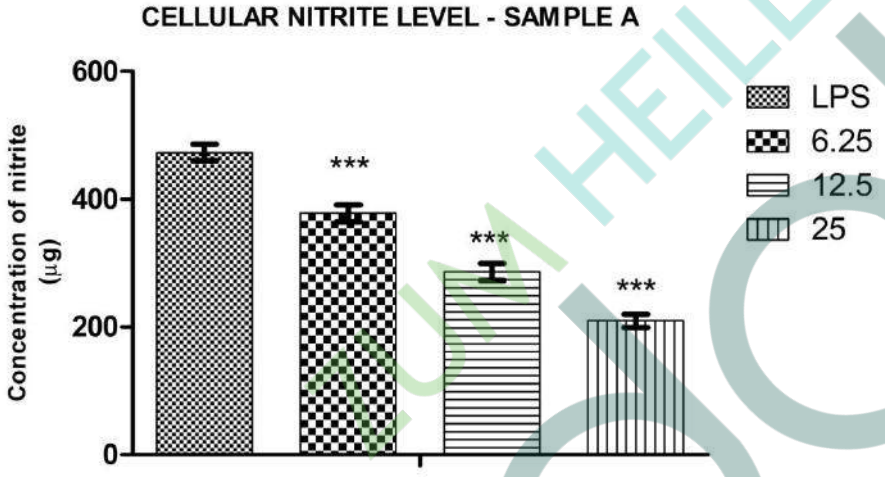
Table Analyzed	Sample A				
One-way analysis of variance					
P value	< 0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	4				
F	243.9				
R squared	0.9892				

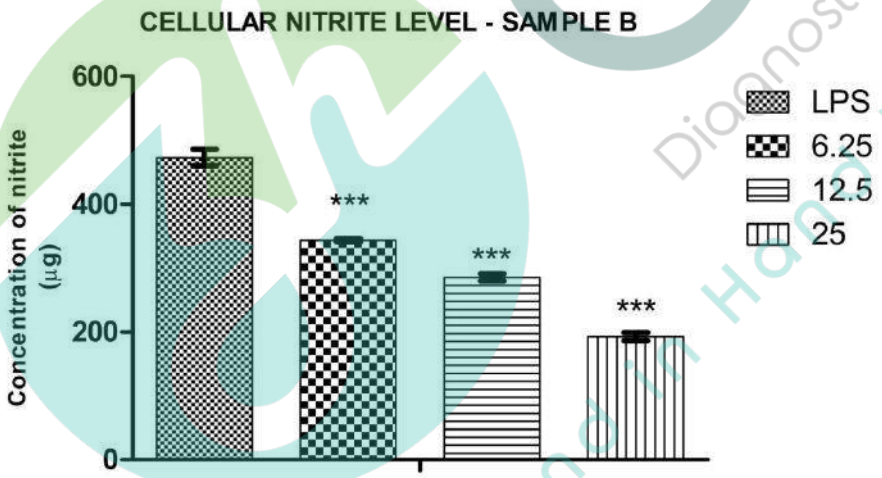
ANOVA Table	SS	df	MS		
Treatment (between columns)	116900	3	38980		
Residual (within columns)	1279	8	159.8		
Total	118200	11			
Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
LPS vs 6.25	94.88	9.191	Yes	***	65.15 to 124.6
LPS vs 12.5	186.8	18.10	Yes	***	157.1 to 216.5
LPS vs 25	263.3	25.51	Yes	***	233.6 to 293.1

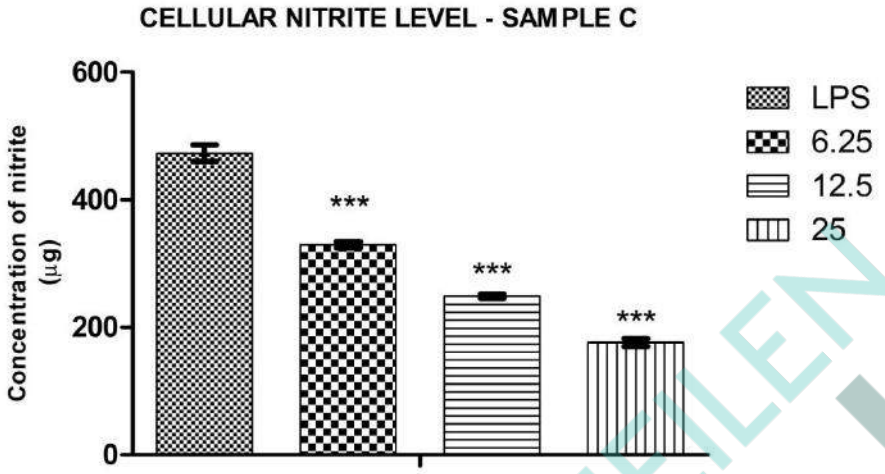
Table Analyzed	Sample B				
One-way analysis of variance					
P value	< 0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	4				
F	635.8				
R squared	0.9958				
ANOVA Table	SS	df	MS		
Treatment (between columns)	123700	3	41220		
Residual (within columns)	518.6	8	64.83		
Total	124200	11			
Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
LPS vs 6.25	129.5	19.70	Yes	***	110.6 to 148.5
LPS vs 12.5	187.4	28.51	Yes	***	168.5 to 206.4
LPS vs 25	280.0	42.59	Yes	***	261.1 to 298.9

Table Analyzed	Sample C				
One-way analysis of variance					
P value	< 0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	4				
F	773.4				
R squared	0.9966				
ANOVA Table	SS	df	MS		
Treatment (between columns)	145500	3	48510		

Residual (within columns)	501.8	8	62.73		
Total	146000	11			
Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
LPS vs 6.25	143.4	22.17	Yes	***	124.8 to 162.0
LPS vs 12.5	224.1	34.65	Yes	***	205.4 to 242.7
LPS vs 25	296.7	45.88	Yes	***	278.0 to 315.3







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