

ANALYSIS OF O⁶-ALKYLGUANINE IN RAT BLOOD DURING RECURRENT ORAL ADMINISTRATION OF CYCLOPHOSPHAMIDE

Yahdiana Harahap¹, Santi Purnasari¹, Hening Herawati²,
Novita Sari¹

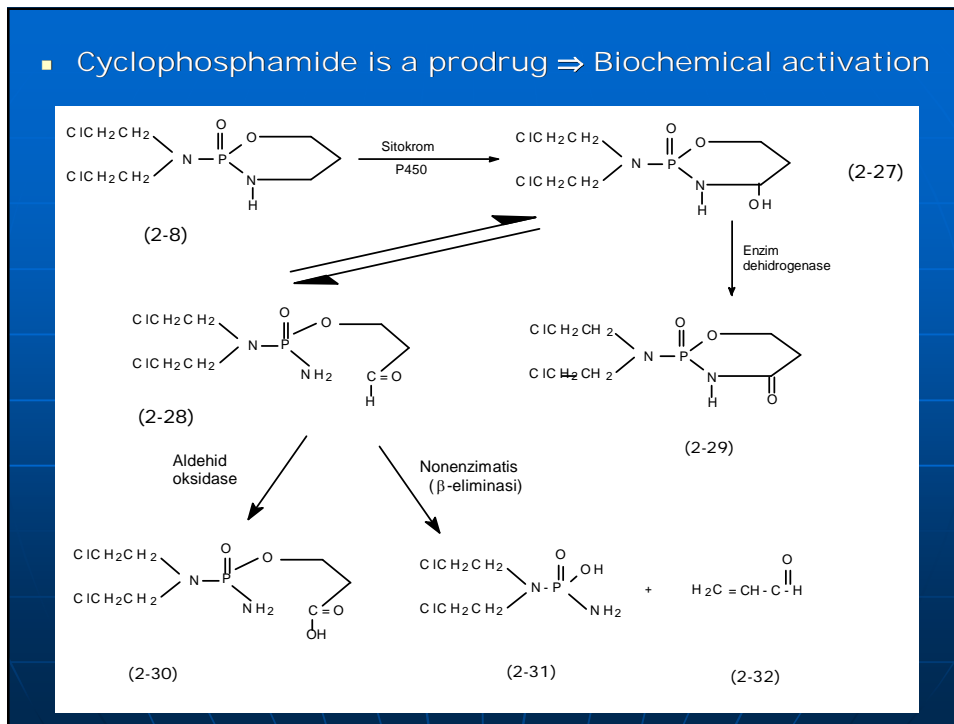
¹ Department of Pharmacy Faculty Mathematics and Natural
Science University of Indonesia

² Dharmais Cancer Hospital

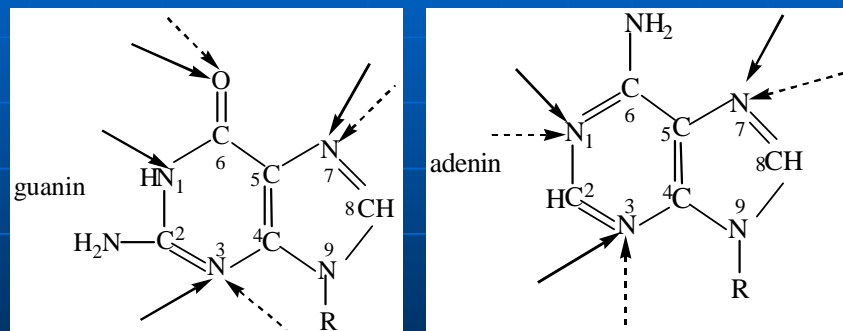
INTRODUCTION

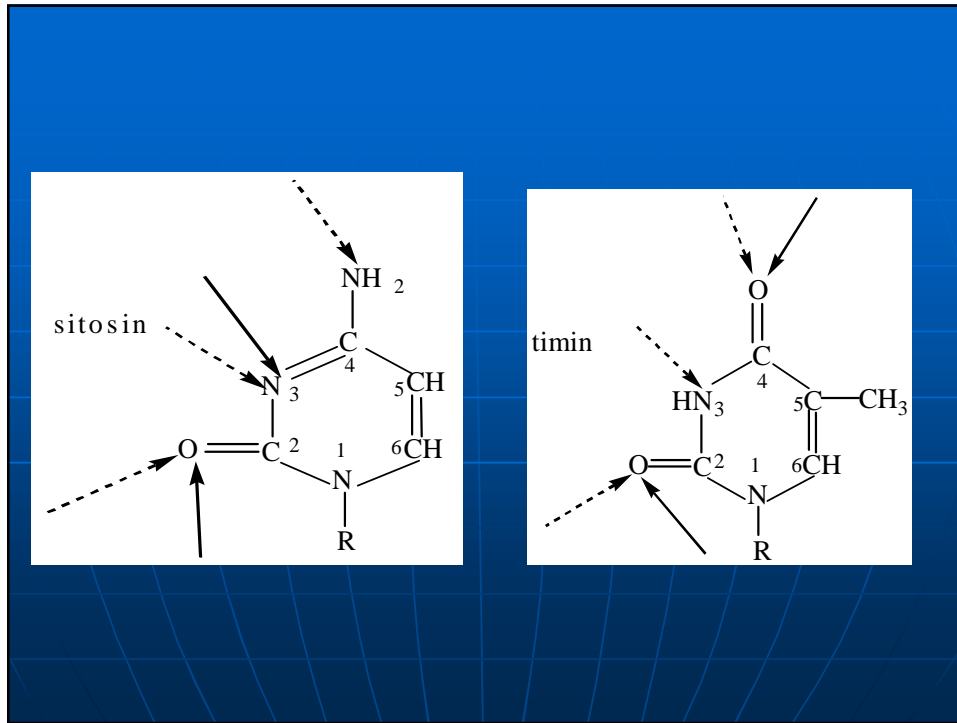
- Cyclophosphamide (Alkylating agents) ⇒ have long been used for cancer therapy due to its effectiveness to destroy cancer cells ⇒ direct reaction with DNA through alkylating process → will cause DNA to lose its function to replicate.
- Cyclophosphamide dosage: 40-50mg/kg bw or 750 mg/m² ⇒ converted to rat 15 mg/ 200 grams.

■ Cyclophosphamide is a prodrug ⇒ Biochemical activation



Cyclophosphamide ⇒ Mustard Phosphoramidate ⇒ react with the nucleophiles in the DNA ⇒ inhibit replication of cancer cell





- *Adduct (additional product) ⇒ covalent substance* which was formed on the reaction of alkylating agent (as electrophile) with DNA base (as nucleophile).

- Unselective reaction \Rightarrow cancer cell
 \Rightarrow healthy cell

\Rightarrow *monofunctional and bifunctional adducts*

- EPIDEMI OLOGY STUDY
(IARC) \rightarrow SECONDARY PRIMER
CANCER

Previous Research
 \Rightarrow Mutagenicity \Leftrightarrow O⁶-alkylguanine
Cytotoxicity \Leftrightarrow N⁷-alkylguanine

ALKYLATING THE LIVING CELL \rightarrow DNA DAMAGE

Mutagenesis

Cancer

Cytotoxicity

Cancer Therapy

- Testing on animal \Rightarrow Carcinogenic after oral or parenteral \longrightarrow benign & malignant tumor on many organs including bladder (Weisberger *et al.*, 1975)
- Epidemiology Study \Rightarrow bladder cancer (Seo *et al.*, 1985) and acute nonlymphocytic leukemia (Portugal *et al.*, 1979)
- From 1349 patients of non malignant disease who received cyclophosphamide therapy for 3 months \Rightarrow 11 times increase of Non Hodgkin Lymphoma in the patient (Kinlen, 1985)

Research Objective

- To analyse DNA adduct O⁶-Alkylguanine on the rat's blood during recurrent oral administration of Cyclophosphamide.

METHODOLOGY

- Material:

O⁶-metilguanine, N⁷-metilguanine, N³-metiladenine, guanine, and adenine (Sigma Aldrich); cyclofosfamida (Sigma Aldrich); ammonia 25 % (Merck); format acid (Merck); methanol (Merck) chloride acid (Malincrodt); Tris (Bromma); aquabidestilata (Wida Wi); male rat Sprague-Dawley; rat food; eter (PT. Bumi Indah); ammonium chloride (Merck); calium carbonate (Merck); Na₂EDTA (Merck); natrium chloride (Merck); sodium duodesil sulfat (SDS) (Merck); natrium hidroxide (Merck); fenol (Merck); chloroform (Merck); ethanol absolute (Merck); ethanol 70%; aquadestilata; dimetilsulfoksida (DMSO) (Merck)

- Tools:

A set of HPLC consisted of column (Supelcosil LC-SCX 5µm, 250 x 4,6 mm), pump (Shimadzu LC-10AD VP), oven column (TC 1900), fluorescence detector (Shimadzu RF-10 A XL), and recorder; syringe 100 µl (Hamilton); penyaring eluen 0,45 µm (Whatman); Refrigerator; Centrifugal tool (Profuge and Lab. Digital Centrifuge DSC-300 SD); timbangan analitik; spuit 5cc; ultrasonic mixer (Elmasonic S 40 H); pipet eppendorf 1000 µl and 100 µl; *blue tip* and *yellow tip*; pH meter (Eutech Instruments pH 510); *sample cup*; *vacutainer* K₃EDTA; *Thermomixer* (Eppendorf); tabung hematocrite (superior); sonde lambung; and glass tools.

Chromatography condition :

High Performance Liquid Chromatography

Column : *Strong Cation Exchange* (Supelcosil LC-SCX, 25 x 4.6 mm)

- **Mobile Phase :** ammonium format-methanol (94:6) with final concentration of ammonium format 30 mM; pH 3,95

Detector : Fluorescence (excitation 300 nm, emission 370 nm)

- **Flow rate :** 1.2 ml/minute.

Administering cyclophosphamide orally on rats

Experimental animal (rat)

Test group

Control group

Test group :

Cyclophosphamide was administered orally with dosage of 15 mg/200 g on rat.

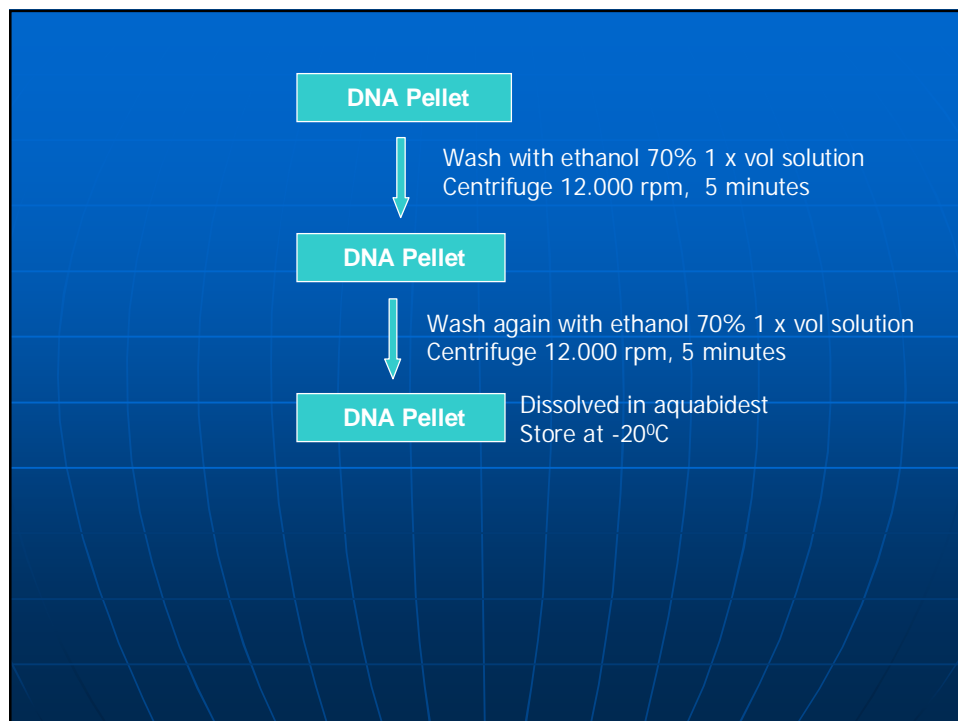
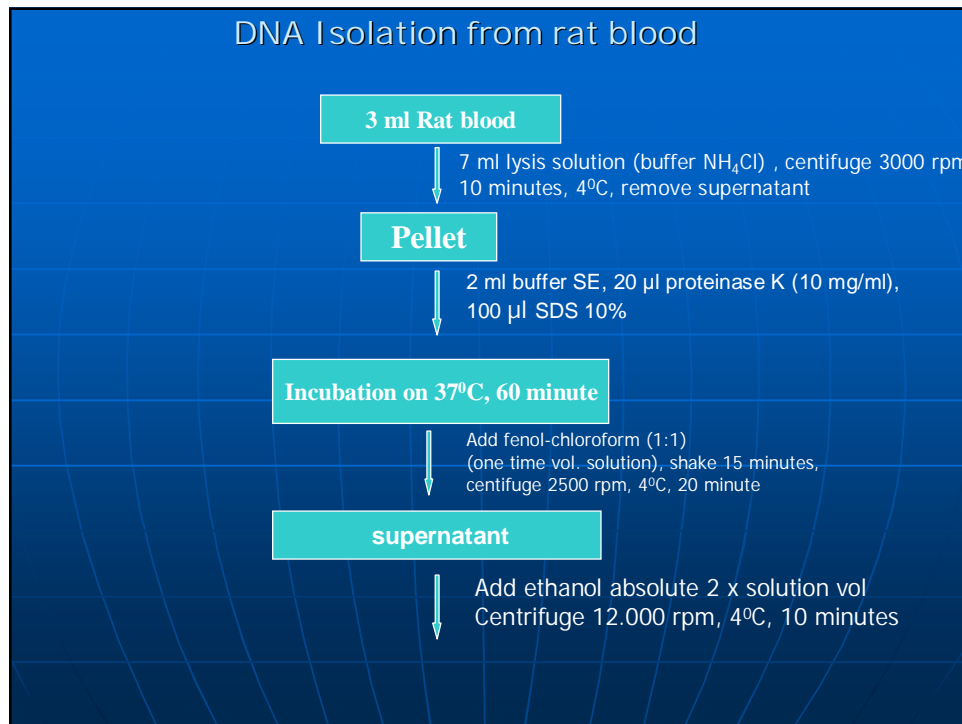
Control group :

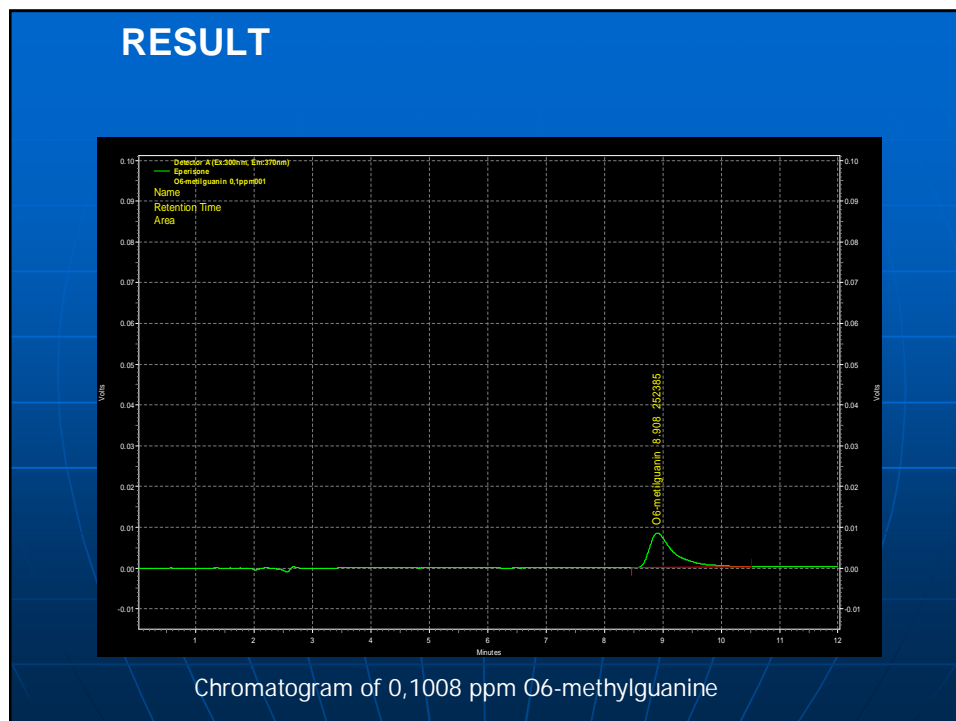
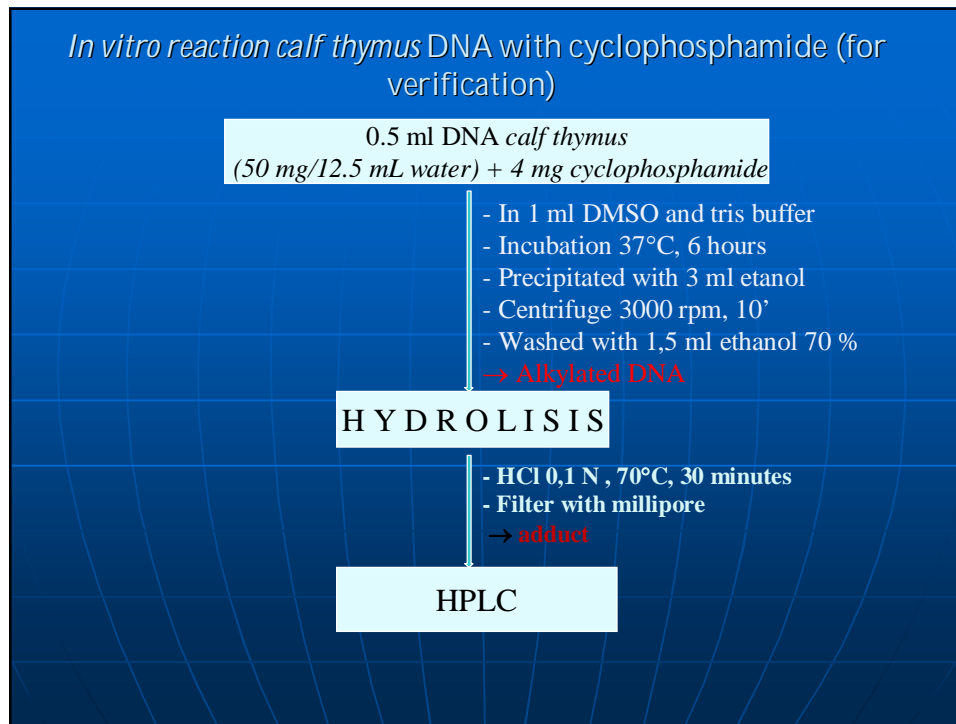
Aquabidest was administered with the appropriate volume.

Drug administering was done for 3 cycles with 2 weeks of drug administering range.

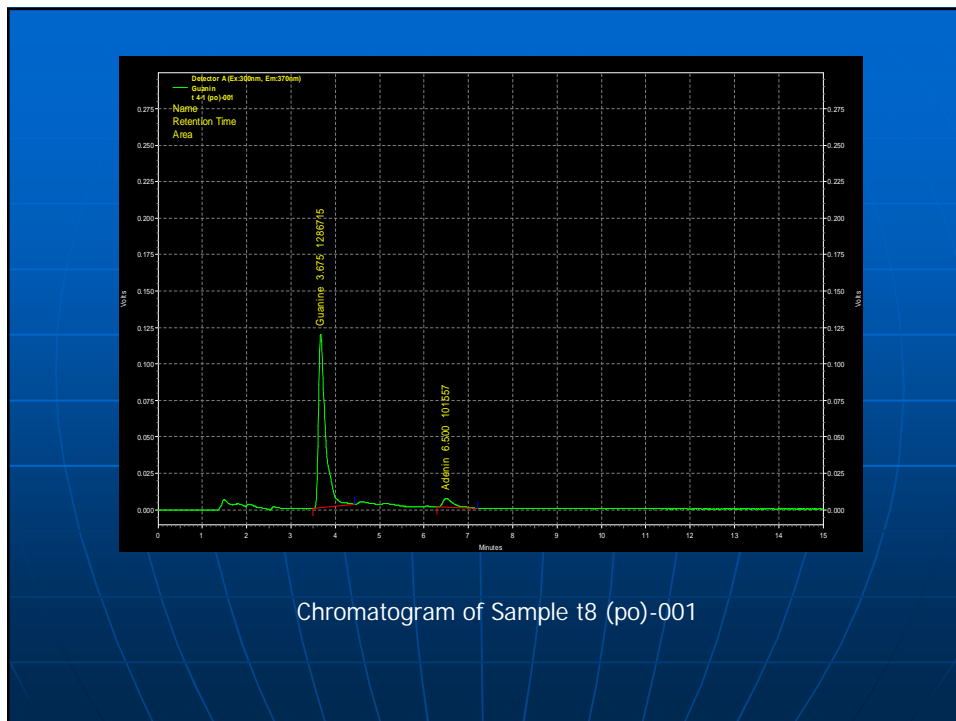
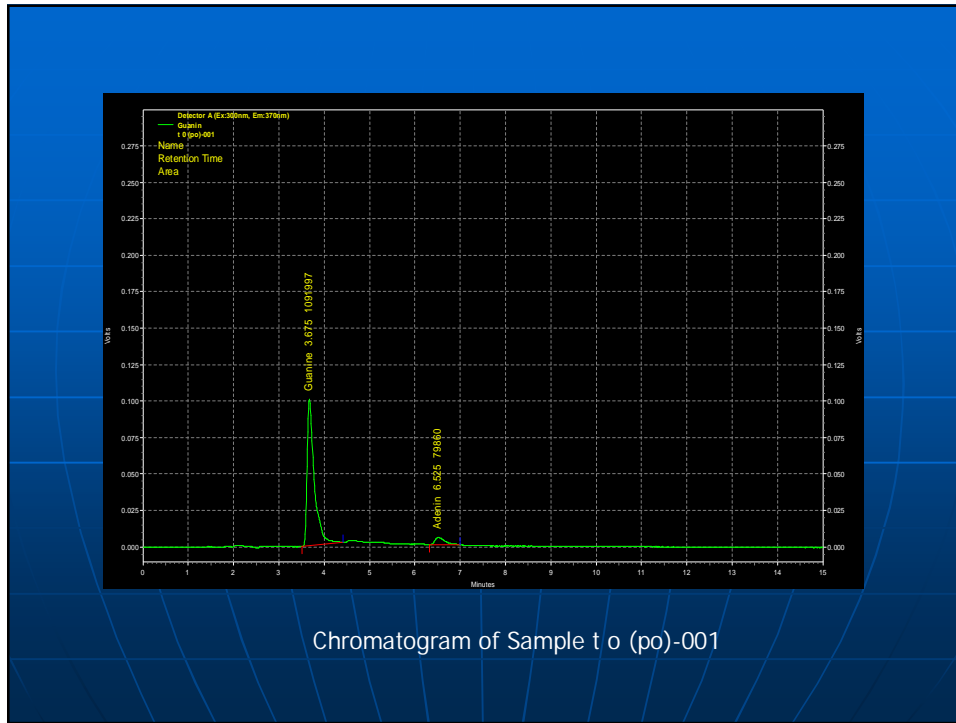


Blood Collecting on Rat through Orbital Angle





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Table 8. Data of Experiment Animal

Experiment Animal	First Cycle					
	0 Hr			8 th Hrs		
	Detected Substance	Retention Time (min)	Area	Detected Substance	Retention Time (min)	Area
Test1	Guanin	3,492	1.842.758	Guanin	3,492	1.943.082
	Adenin	5,725	188.370	Adenin	5,733	207.247
Test2	Guanin	3,492	1.104.563	Guanin	3,492	1.052.863
	Adenin	5,733	120.303	Adenin	5,742	112.417
Test3	Guanin	3,592	1.151.488	Guanin	3,592	1.115.697
	Adenin	6,167	114.691	Adenin	6,175	113.133
Test4	Guanin	3,525	482.132	Guanin	3,517	471.357
	Adenin	5,950	46.245	Adenin	5,95	46.484
Test5	Guanin	3,508	685.796	Guanin	3,517	581.811
	Adenin	5,925	69.293	Adenin	5,942	60.297
Test6	Guanin	3,525	740.753	Guanin	3,517	419.712
	Adenin	5,958	68.875	Adenin	5,933	39.145
Test7	Guanin	3,508	826.976	Guanin	3,517	527.837
	Adenin	5,917	67.298	Adenin	5,95	52.064
Test8				Guanin	3,517	233.653
				Adenin	5,958	16.528
Test9	Guanin	3,508	705.131	Guanin	3,517	673.116
	Adenin	5,950	71.782	Adenin	5,933	67.620
Test10						

Experiment Animal	Second cycle					
	0 hour			8 th hour		
	Detected Substance	Retention Time (min)	Area	Detected Substance	Retention Time (min)	Area
Test 1						
Test 2	Guanin	3,592	1.525.202	Guanin	3,583	1.142.174
	Adenin	6,158	141.854	Adenin	6,142	85.116
Test 3						
Test4	Guanin	3,567	629.732	Guanin	3,558	774.552
	Adenin	6,142	56.874	Adenin	6,108	65.001
Test 5	Guanin	3,583	529.053	Guanin	3,583	384.948
	Adenin	6,175	43.762	Adenin	6,142	29.125
Test 6	Guanin	3,575	653.010	Guanin	3,575	154.994
	Adenin	6,133	67.052	Adenin	6,183	10.334
Test 7	Guanin	3,558	575.968	Guanin	3,567	521.009
	Adenin	6,117	45.586	Adenin	6,133	33.674
Test 8	Guanin	3,558	349.828	Guanin	3,567	1.352.972
	Adenin	6,15	26.585	Adenin	6,092	131.674
Test 9	Guanin	3,558	351.994	Guanin	3,55	1.026.638
	Adenin	6,117	29.460	Adenin	6,075	99.063
Test 10						

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Experiment Animal	Third Cycle					
	0 hour			8 th hour		
	Detected Substance	Retention Time (min)	Area	Detected Substance	Retention Time (min)	Area
Test 1						
Test 2	Guanin	3,483	779.101	Guanin	3,483	476.486
	Adenin	5,817	57.519	Adenin	5,817	35.415
Test 3						
Test 4						
Test 5						
Test 6						
Test 7	Guanin	3,425	636.122	Guanin	3,425	110.776
	Adenin	5,55	45.488			
Test 8						
Test 9						
Test 10						

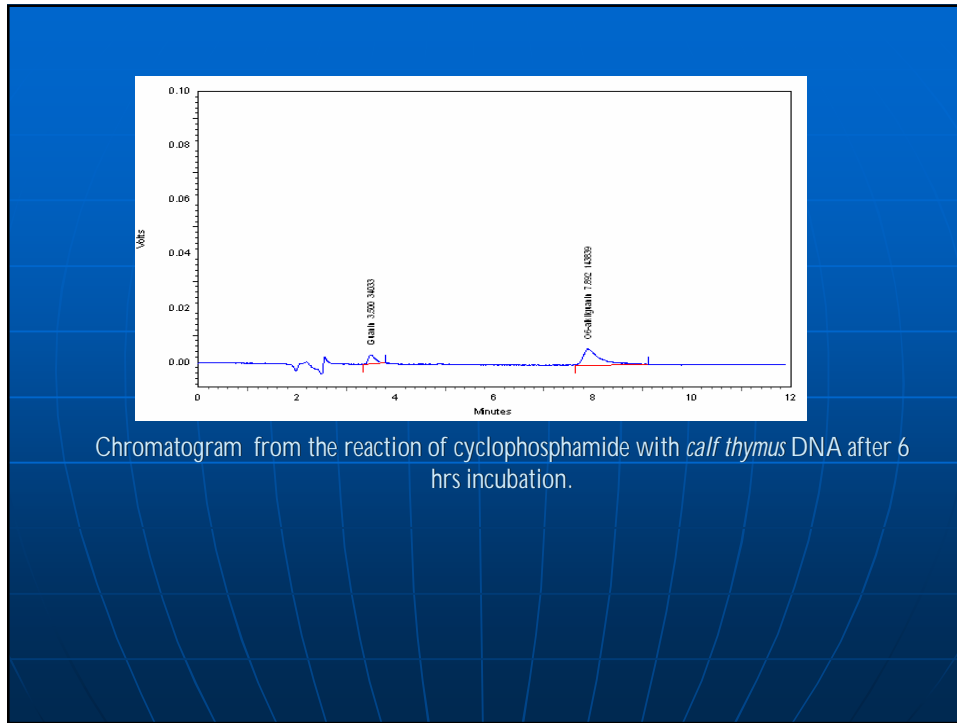
Table 9. Control animal sample data

Experiment Animal	First cycle					
	0 hour			8 th hour		
	Detected Substance	Retention Time (min)	Area	Detected Substance	Retention Time (min)	Area
Control 1	Guanin	3,492	873.814	Guanin	3,492	524.436
	Adenin	5,750	87.315	Adenin	5,758	52.820
Control 2	Guanin	3,600	1.583.895	Guanin	3,592	1.098.816
	Adenin	6,175	156.103	Adenin	6,175	111.262
Control 3	Guanin	3,600	454.113	Guanin	3,6	1.102.908
	Adenin	6,200	51.209	Adenin	6,183	106.744
Control 4						
Control 5	Guanin	3,508	1.265.266	Guanin	3,483	494.664
	Adenin	5,925	130.433	Adenin	5,883	47.288
Control 6				Guanin	3,558	306.507
				Adenin	6,083	20.652
Control 7	Guanin	3,567	590.984	Guanin	3,567	402.694
	Adenin	6,117	57.902	Adenin	6,117	38.227
Control 8	Guanin	3,517	666.511			
	Adenin	5,942	64.806			
Control 9						
Control 10	Guanin	3,567	742.025	Guanin	3,567	942.319
	Adenin	6,092	81.593	Adenin	6,092	95.807

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Experiment Animal	Second cycle					
	0 hour			8 th hour		
	Detected Substance	Retention Time (min)	Area	Detected Substance	Retention Time (min)	Area
Control 1	Guanin	3,583	196.090	Guanin	3,592	558.151
	Adenin	6,133	16.452	Adenin	6,175	51.524
Control 2	Guanin	3,558	640.910	Guanin	3,567	780.212
	Adenin	6,083	60.769	Adenin	6,092	72.623
Control 3	Guanin	3,558	1.040.994	Guanin	3,558	608.020
	Adenin	6,058	96.280	Adenin	6,067	52.648
Control 4						
Control 5	Guanin	3,5	548.843	Guanin	3,5	1.324.089
	Adenin	5,933	58.056	Adenin	5,9	143.135
Control 6						
Control 7	Guanin	3,5	1.599.883	Guanin	3,5	1.703.614
	Adenin	5,9	171.315	Adenin	5,908	136.444
Control 8	Guanin	3,5	786.811	Guanin	3,5	1.642.681
	Adenin	5,933	56.199	Adenin	5,908	121.350
Control 9						
Control 10	Guanin	3,517	783.155	Guanin	3,508	1.479.860
	Adenin	5,958	62.594	Adenin	5,917	104.197

Experiment Animal	Third Cycle					
	0 hour			8 th hour		
	Detected Substance	Retention Time (min)	Area	Detected Substance	Retention Time (min)	Area
Control 1	Guanin	3,467	636.148	Guanin	3,458	833.747
	Adenin	5,783	45.452	Adenin	5,767	61.580
Control 2	Guanin	3,483	648.350	Guanin	3,483	791.449
	Adenin	5,817	50.025	Adenin	5,817	59.110
Control 3	Guanin	3,483	777.704	Guanin	3,467	775.467
	Adenin	5,817	56.215	Adenin	5,792	61.965
Control 4						
Control 5	Guanin	3,425	827.189	Guanin	3,425	1.116.225
	Adenin	5,542	57.523	Adenin	5,533	78.387
Control 6						
Control 7	Guanin	3,417	1.030.601	Guanin	3,417	1.216.623
	Adenin	5,542	76.379	Adenin	5,533	87.452
Control 8	Guanin	3,417	968.968	Guanin	3,417	736.677
	Adenin	5,533	70.810	Adenin	5,525	52.178
Control 9						
Control 10						



Conclusion

- In this research, after three series of cyclophosphamide administrations, O⁶-Alkylguanine had not been found in rat blood.
- However during *in vitro* reaction as verification, it yield O⁶-methylguanine after 6 hours incubation

