A Toxicity Study of LEAF Consumption

FINAL REPORT

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CONTENTS

1. Objective

The purpose of this study was to investigate the possible toxicity of LEAF herbal mix consumption in female vervet monkeys (*Chlorocebus aethiops*), by determining certain biochemical, haematological, physiological and physical variables. These variables reflect liver, kidney, muscle, respiratory, intestinal, bone, and general biological function.

2. Ethical approval

The study was approved by the Ethics Committee for Research on Animals (ECRA) of the Medical Research Council (Project No. 115).

3. Abbreviations

AST	Aspartate Aminotransferase
ALT	Alanine Aminotransferase
ALP	Alkaline Phosphatase
Ca	Calcium
CK	Creatine Kinase
Cl	Chloride
Fe	Iron
GGT	Gamma Glutamyl Transferase
Hb	Haemoglobin
Hct	Haematocrit
HDL-C	High Density Lipoprotein Cholesterol
K	Potassium
LDH	Lactate Dehydrogenase
P	Phosphate
LDL-C	Low Density Lipoprotein Cholesterol
MCV	Mean Corpuscular Volume
MCH	Mean Corpuscular Haemoglobin
MCHC	Mean Corpuscular Haemoglobin Concentration
Mg	Magnesium
Na	Sodium
RBC	Red Blood Cells
RDW	Red Cell Distribution Width
WBC	White Blood Cells

4. Materials and methods

4.1. Nonhuman primates, environment and housing

All individuals selected for this project were healthy adult female vervet monkeys, which were identified with numbers in ink tattoo. Additionally, all cages were marked according to individual, group designation, and experiment number. They were maintained in the Primate Unit of the Diabetes Research Group of the MRC under identical housing conditions. The closed indoor environment was maintained at 25 – 27 °C, a humidity of 45%, about 15-20 air changes/hour and a photoperiod of 12h. A maintenance diet, which consists of 120g of stiff maize porridge, containing micro- and macronutrient supplementation, was fed throughout the study and water was available *ad lib* via an automatic watering device. All individuals were housed singly during the study but had regular access to exercise cages and environmental enrichment.

4.2. Treatments

4.2.1. Plant material

The herbal preparation coded LEAF was a mixture of Hypoxis/African potato, Milk thistle, Beta-sitosterol/plant sterols and Spirulina (*Spirulina plantensis*). The two plants, hypoxis and milk thistle, were sourced from farms in Kwa Zulu-Natal and Free State, respectively. The source of Beta-sitosterol and Spirulina was not supplied to the Primate Unit. All plant materials were washed to remove all debris and air dried out of direct sunlight. Each plant was homogenously milled into a powder form and sterilized by irradiation at 18kGY for not more than 24 hours by the company HEPRO, Montague Gardens, Cape Town. The powders were tested before and after irradiation for bacterial and fungal contamination by Kirsch Pharma SA, Isando, Johannesburg (for Milk thistle); and Warren Chem C/O, Sandringham, Johannesburg (for Beta-sitosterol and Spirulina) [See Appendix VIII, page 78). Detailed information on testing and irradiation of hypoxis for bacterial and fungal contamination was not supplied to the Primate Unit. The plant materials were mixed to the correct proportions, as in table 1, at the Primate Unit, where the study was conducted. The same batch of plant material was used for the entire study. The daily recommended dose provided in table 2 (page 4) is traditionally used by people.

4.2.2. Dose

Sixteen female vervet monkeys were randomly divided into four groups of four individuals each and allocated according to table 2.

Table 1. Proportions of plant materials in LEAF

Name of plant material	Daily dose(mg)/ 65kg bodyweight human	Daily dose (mg/kg bodyweight)
Spirulina	200	3.1
African potato	200	3.1
Milk thistle	60	0.9
Beta sitosterol	60	0.9
Total dose(LEAF)	**520**	**8**

Table 2. Group allocations and treatments

Group	Total herbal mix concentration
1	8 mg/kg bodyweight (recommended dose) referred to as low dose
2	40 mg/kg bodyweight (5x recommended dose) referred to as medium dose
3	200 mg/kg bodyweight (25x recommended dose) referred to as high dose
4	Control (maintenance diet)

4.2.3. Duration of treatments

The treatment period was three months, from January 20th, 2004 to April 20th ,2004.

4.3. Administration of LEAF

All individuals in Groups 1-3 received their respective doses of LEAF mixed into the maintenance diet, Group 4 received only the maintenance diet.

Food consumption was monitored daily, and recorded once every two weeks on the same day. The latter was then used to calculate monthly intake.

4.4. Dosing schedule

The diet containing LEAF was fed daily at 7H00 throughout the treatment period and the same feeding times were adhered to.

4.5. Clinical monitoring

Once a month during the study period, each individual was sedated with Ketamine hydrochloride (Anaket-V, Centaur Labs, Bayer Animal Health, Isando) at 10mg/kg bodyweight intramuscularly. For clinical evaluation and blood sampling, all vervet monkeys were fasted overnight. Bodyweight, body temperature, as well as respiratory rate were recorded, and heart rate and blood pressures were measured using a Dinamap XL vital signs monitor with a neonatal blood pressure cuff #4. At the time of physical and physiological measurements, blood samples were collected by femoral venipuncture into SST, EDTA and sodium fluoride tubes. The following haematological and biochemical tests were then conducted at an accredited commercial pathology laboratory (Pathcare).

Haematology: RBC, Hct, Hb, MCV, MCH, MCHC, RDW, WBC, neutrophils, eosinophils, basophils, lymphocytes, monocytes and platelets were determined with a Coulter STAK S.

Clinical Biochemistry: bilirubin (total and direct), AST, ALT, ALP, GGT, LDH, CK, total proteins, albumin, globulin, cholesterol (total, LDL-C, HDL-C), glucose, urea, creatinine, Na, K, Cl, Ca, Mg, P and total plasma Fe were determined with a Technicon autoanalyzer.

Note: for definition of abbreviations see section 3.

Urine was collected once/month by means of a funnel placed under each cage, and analysed with urine test strips (UriCheck, RapiMED Diagnostics, South Africa) for leucocytes, nitrite, urobilinogen, protein, pH, blood, specific gravity, ketones, bilirubin and glucose.

Note: Except for two quantitative variables (pH and specific gravity), urinalysis data are qualitative.

4.6. Observations

All individuals were observed daily according to the sample checklists provided in Appendix VII and any patterns of changes in behaviour (e.g. depressed, fearful, unresponsive, confused, excited, irritable, and aggressive) were noted. Apart from clinical signs such as loss of appetite and diarrhoea, the following criteria were used to determine well-being: coat condition, posture, locomotion, activity, vocalisation and activity in the exercise cage.

4.7. Statistics

All variables were analysed by the MRC Biostatistics Unit utilizing the SAS Version 8 statistical package with Repeated Measures Analysis of Variance; $P<0.05$ was considered significant. Statistics for changes from baseline were generated for time effect, group effect, time - group interactions and differences between each treatment group and the controls.

5. Results

5.1. Preliminary considerations

- Results and observations are reported and interpreted mainly in terms of possible treatment effects and not biological variation.
- The results in a study such as this do not preclude individual susceptibility and response to the consumption of herbal medicines or any other medicinal compound.
- LEAF was not tested in pregnant or young animals and the results cannot be extrapolated to these groups.
- The study design does not allow for speculation regarding carcinogenicity, developmental toxicity, and chronic toxicity.

5.2. Compliance

Habituation to the LEAF took 7 days, which is a normal period required for similar studies with other plants. The decrease in consumption of food containing LEAF was slightly higher in Group 2 compared to others (table 3).

Table 3. Average consumption of food containing LEAF (% mean ± sd)

Group	Baseline	Week 4	Week 8	Week 12
1	100.00 ± 0.00	97.50 ± 5.00	94.50 ± 7.55	93.25 ± 4.99
2	96.25 ± 7.50	83.50 ± 19.12	84.25 ± 23.64	87.75 ± 13.23
3	92.25 ± 9.18	96.25 ± 7.50	93.50 ± 8.54	85.00 ± 11.92
4*	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00

*Food consumption of maintenance diet only.

5.3. General observations

Apart from instances of poor appetite and vomition (table 4), LEAF consumption did not lead to any change in behaviour, neuromotor function or general wellbeing.

Table 4. Number of instances of reduced food consumption and vomition during the 90 day period

Group	Reduced appetite		Vomition	
	Instance(s)	Monkey number	Instance(s)	Monkey number
1	-	-	2	181
2	6 3	108 110	2 1	108 110

3	3	234	1	234
4	-	-	-	-

5.4. Haematology

Graphs and profiles of all means and standard deviations (±SD) are provided in appendices I (pages 14 - 28) and II (pages 29 - 32), respectively. Statistically significant changes were recorded in certain parameters, but were not considered to be due to a treatment effect (e.g. changes in controls rather than treated groups).

- A statistically significant time effect in RBC, Hb, Hct, MCHC, RDW, Platelets, WBC and neutrophils was clinically insignificant and not considered to be due to the treatment.
- A statistically significant group effect in MCV (controls against Group 2) was clinically insignificant and not considered a treatment effect.
- All statistically significant results generated for time-group interactions were not clinically significant and not considered a treatment effect, except possibly for lymphocytes in Group 3.

Table 5. Statistically significant data generated for haematological variables

Variable	Time effect	Group effect	Control vs.	Time – group interaction	Control vs.
RBC	0.0003	-	-	-	-
Hb	0.0108	-	-	-	-
Hct	0.0130	-	-	-	-
MCV	-	0.0205	Gr 2: 0.0401	-	-
MCH	-	-	-	-	-
MCHC	0.0003	-	-	-	Gr 2: 0.0448 Gr 3: 0.0448
RDW	<0.0001	-	-	-	Gr 2: 0.0337 Gr 3: 0.0226
Platelets	0.0120	-	-	-	-
WBC	0.0054	-	-	-	-
Neutrophils	0.0369	-	-	-	-
Lymphocytes	-	-	-	0.0084	Gr 1: 0.0299 Gr 2: 0.0464 Gr 3: 0.0072
Monocytes	-	-	-	-	-

Eosinophils	-	-	-	-	-
Basophils	-	-	-	-	Gr 1: 0.0068

5.5. Biochemistry

Graphs and profiles of all means and standard deviations (±SD) are provided in appendices III (pages 33 - 56) and IV (pages 57 - 62), respectively.

Significant changes were recorded in certain parameters but were not considered due to a treatment effect (e.g. changes in controls rather than the treated groups, specific profiles, etc.) and are therefore not discussed.

- Statistically significant time-group interactions generated between Group 2 and the controls, increases in ALT for both Groups 2 and 3 were of clinical significance, since the mean values exceeded to a large extent reference values established in the colony and the controls.
- Statistically significant time-group interactions recorded for each treated group when compared to the controls, were not due to a treatment effect. All groups started at similar concentrations and declined to almost identical levels at week 12.
- Statistically significant differences in total proteins and albumin between controls and Groups 1 and 2, respectively, as well as in globulin between controls and Group 1, were of no clinical significance.
- Statistically significant differences generated for CK between controls and Group 3 were of no clinical significance.
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Table 6. Statistically significant data generated for biochemical variables

Variable	Time interaction	Group interaction	Control vs.	Time – group interaction	Control vs.
GGT	0.0079	-	-	-	-
Total bilirubin	-	-	-	-	-
Direct bilirubin	0.0010	-	-	-	-
AST	-	-	-	-	-
ALT	0.0006	-	-	0.0306	Gr 2: 0.0120
ALP	<0.0001	-	-	-	-
Cretinine	0.0002	-	-	0.0035	Gr 1: 0.0036 Gr 2: 0.0004 Gr 3: 0.0462
Urea	-	0.0316	-	-	-
Glucose	-	0.0134	Gr 1: 0.0042 Gr 2: 0.0152 Gr 3: 0.0053	-	Gr 2: 0.0164 Gr 3: 0.0327
Total Proteins	0.0031	-	Gr 1: 0.0386	0.0046	Gr 1: 0.0083 Gr 2: 0.0056
Albumin	<0.0001	-	-	0.0033	Gr 1: 0.0016

					Gr 2: 0.0005
Globulin	0.0285	-	-	-	Gr 1: 0.0132
Total Cholesterol	-	-	-	-	-
HDL-C	-	-	-	-	-
LDL-C	-	0.0498	Gr 1: 0.0103	-	-
Ca	0.0006	-	-	-	Gr 1: 0.0406
Mg	<0.0001	-	-	-	-
P	0.0007	0.0147	Gr 1: 0.0168 Gr 2: 0.0031	-	Gr 1: 0.0499
Na	<0.0001	-	-	-	-
K	<0.0001	-	-	-	-
Cl	<0.0001	-	-	-	-
CK	-	-	-	-	Gr 3: 0.0194
LD	0.0002	-	Gr 1: 0.0293	-	-

5.6. Physical and physiological variables

Graphs and profiles of all means and standard deviations (±SD) are provided in appendices III (page 63 - 70) and IV (pages 71 - 73).

- Statistically significant differences generated for bodyweight, pulse, respiratory rate and body temperature between controls and Group 3 and 1, were of no clinical significance.

Table 7. Statistically significant data generated for physiological variables

Variable	Time effect	Group effect	Control vs.	Time – group interaction	Control vs.
Bodyweight	<0.0001	-	-	-	Gr 3: 0.0433
Diastolic Pressure	-	-	-	-	-
Systolic Pressure	-	-	-	-	-
MAP	-	-	-	-	-
Pulse	-	-	-	0.0284	Gr 3: 0.0109
Body temperature	-	-	-	-	Gr 1: 0.0256
Respiratory rate	-	-	Gr 3: 0.0470	-	-

5.7. Urine analysis

- No changes consistent with a treatment effect could be observed in any group. Means and standard deviations for urine pH and specific gravity are provided in tables 8 and 9, respectively.

Table 8. Urine pH

Group		Baseline	Week 4	Week 8	Week 12
1	Mean	5.25	7.50	5.75	6.00
	SD	0.50	1.22	0.50	0.82
2	Mean	5.50	7.63	5.75	5.50
	SD	0.58	0.75	0.50	0.58
3	Mean	5.25	7.25	5.50	5.25
	SD	0.50	0.87	0.58	0.50
Controls	Mean	5.75	7.13	5.75	5.75
	SD	0.50	0.63	0.50	0.50

Table 9. Urine specific gravity

Group		Baseline	Week 4	Week 8	Week 12
1	Mean	1.00	1.01	1.01	1.01
	SD	0.00	0.01	0.01	0.00
2	Mean	1.02	1.01	1.00	1.02
	SD	0.01	0.00	0.01	0.00
3	Mean	1.02	1.01	1.03	1.03
	SD	0.01	0.00	0.02	0.01
Controls	Mean	1.02	1.01	1.02	1.02
	SD	0.01	0.00	0.01	0.01

6. Conclusions

Note: These conclusions refer to adult female vervet monkeys consuming LEAF for three months.

- Consumption of food containing LEAF by individuals in Groups 1-3 was within targeted doses.
- Consumption of LEAF was not associated with deterioration in physical condition and/or health.
- Except for possible effects on lymphocytes,LEAF was not associated with clinical changes in any other haematological parameters determined in this study.
- A possible effect of LEAF on liver function was observed with increases in plasma ALT concentrations in Groups 2 and 3. However, this was only statistically significant for Group 2.
- Although there were statistically significant differences generated for some biochemical parameters, their concentrations were, however, within normal limits determined for the colony.
- None of the physical and physiological parameters deviated significantly between baseline and the end of the study.

Unit abbreviations for Appendices I – VI

%	percentage
°C	degrees Celsius
beats/min	beats/minute
breaths/min	breaths/minute
fl	femtolitre
g/dL	gram/decilitre
g/L	gram/litre
u/L	units/litre
kg	kilogram
mg/kg	milligram/kilogram
mm/Hg	millimetre Mercury
mmol/L	millimol/litre
pg	picogram
umol/L	micromol/litre

Appendix I: Haematology (Graphs)

Appendix II: Haematology (Means and standard deviations)

Red Blood Cells ($X10^{12}/L$)

Group		Baseline	Week 4	Week 8	Week 12
8 mg/kg bodyweight	Mean	5.11	5.34	5.12	5.29
	SD	0.19	0.25	0.16	0.17
40 mg/kg bodyweight	Mean	5.49	5.48	5.26	5.44
	SD	0.35	0.40	0.33	0.25
200 mg/kg bodyweight	Mean	5.33	5.34	5.30	5.25
	SD	0.08	0.35	0.35	0.36
Controls	Mean	5.22	5.46	5.27	5.31
	SD	0.15	0.26	0.18	0.16

Haematocrit (%)

Group		Baseline	Week 4	Week 8	Week 12
8 mg/kg bodyweight	Mean	46.25	48.50	47.25	48.00
	SD	2.22	1.73	2.22	1.83
40 mg/kg bodyweight	Mean	48.25	48.00	46.25	47.25
	SD	2.99	2.94	2.99	0.96
200 mg/kg bodyweight	Mean	47.00	47.00	47.00	46.50
	SD	0.00	2.58	2.94	2.89
Controls	Mean	46.00	48.25	46.75	46.50
	SD	2.16	2.22	1.50	1.29

Haemoglobin (g/dl)

Group		Baseline	Week 4	Week 8	Week 12
8 mg/kg bodyweight	Mean	12.90	13.75	13.30	13.73
	SD	0.56	0.47	0.41	0.44
40 mg/kg bodyweight	Mean	13.73	13.65	13.10	13.70
	SD	0.88	0.98	0.82	0.36
200 mg/kg bodyweight	Mean	13.35	13.45	13.38	13.45
	SD	0.19	0.84	0.68	0.66
Controls	Mean	13.05	13.53	13.35	13.38
	SD	0.45	0.77	0.59	0.44

Mean Corpuscular Volume (fl)

Group		Baseline	Week 4	Week 8	Week 12
8 mg/kg bodyweight	Mean	90.50	90.75	91.75	90.75
	SD	1.29	2.63	2.06	2.06
40 mg/kg bodyweight	Mean	88.25	87.50	85.75	86.75
	SD	2.63	2.65	4.92	2.22
200 mg/kg bodyweight	Mean	87.75	88.00	89.00	88.50
	SD	1.50	2.16	2.45	1.91
Controls	Mean	88.00	88.00	88.50	87.50
	SD	1.63	1.41	1.91	1.73

Mean Corpuscular Haemoglobin (pg)

Group		Baseline	Week 4	Week 8	Week 12
8 mg/kg bodyweight	Mean	25.25	26.00	25.75	25.75
	SD	0.50	0.82	0.50	0.50
40 mg/kg bodyweight	Mean	25.00	25.00	25.00	25.25
	SD	0.82	0.82	1.15	0.50
200 mg/kg bodyweight	Mean	25.25	25.50	25.25	25.75
	SD	0.96	0.58	0.96	0.50
Controls	Mean	25.00	24.75	25.50	25.25
	SD	0.00	0.96	1.00	0.50

Mean Corpuscular Haemoglobin Concentration (g/dl)

Group		Baseline	Week 4	Week 8	Week 12
8 mg/kg bodyweight	Mean	28.00	28.50	28.50	29.00
	SD	0.00	0.58	0.58	0.00
40 mg/kg bodyweight	Mean	28.25	28.75	28.25	29.00
	SD	0.50	0.50	0.50	0.00
200 mg/kg bodyweight	Mean	28.75	28.75	28.25	29.00
	SD	0.50	0.50	0.50	0.00
Controls	Mean	28.25	28.00	28.50	29.00
	SD	0.50	0.00	0.58	0.00

Red Cell Distribution Width (%)

Group		Baseline	Week 4	Week 8	Week 12
8 mg/kg bodyweight	Mean	17.48	15.88	15.45	14.70
	SD	1.49	0.93	0.64	0.42
40 mg/kg bodyweight	Mean	16.33	15.40	15.90	15.13
	SD	1.10	0.62	0.57	0.93
200 mg/kg bodyweight	Mean	16.68	15.05	15.83	14.85
	SD	0.97	0.61	0.88	0.81
Controls	Mean	17.20	17.03	16.63	15.10
	SD	0.27	0.73	0.91	1.30

Total White Blood Cells ($X10^9/L$)

Group		Baseline	Week 4	Week 8	Week 12
8 mg/kg bodyweight	Mean	3.68	4.70	4.75	5.13
	SD	0.79	0.71	0.42	1.35
40 mg/kg bodyweight	Mean	3.33	4.08	5.10	5.33
	SD	1.45	1.43	1.26	1.97
200 mg/kg bodyweight	Mean	3.63	4.48	5.85	6.65
	SD	0.49	0.43	0.49	1.53
Controls	Mean	3.18	5.40	4.88	6.15
	SD	0.73	1.13	1.85	2.26

Neutrophils ($X10^9/L$)

Group		Baseline	Week 4	Week 8	Week 12
8 mg/kg bodyweight	Mean	1.37	1.85	1.89	2.44
	SD	0.32	0.97	0.49	0.94
40 mg/kg bodyweight	Mean	1.08	1.65	2.22	2.38
	SD	0.50	0.97	0.89	1.16
200 mg/kg bodyweight	Mean	1.68	2.03	2.67	3.26
	SD	0.59	0.89	1.07	1.58
Controls	Mean	1.44	2.73	2.59	3.41
	SD	0.46	1.06	1.82	1.90

Eosinophils ($X10^9/L$)

Group		Baseline	Week 4	Week 8	Week 12
8 mg/kg bodyweight	Mean	0.03	0.02	0.01	0.18
	SD	0.04	0.01	0.01	0.18
40 mg/kg bodyweight	Mean	0.04	0.03	0.08	0.12
	SD	0.03	0.02	0.11	0.15
200 mg/kg bodyweight	Mean	0.04	0.06	0.06	0.37
	SD	0.04	0.08	0.06	0.53
Controls	Mean	0.02	0.01	0.06	0.01
	SD	0.03	0.01	0.11	0.01

Basophils ($X10^9/L$)

Group		Baseline	Week 4	Week 8	Week 12
8 mg/kg bodyweight	Mean	0.04	0.08	0.07	0.01
	SD	0.04	0.06	0.07	0.02
40 mg/kg bodyweight	Mean	0.05	0.03	0.03	0.03
	SD	0.07	0.00	0.02	0.01
200 mg/kg bodyweight	Mean	0.02	0.02	0.03	0.02
	SD	0.01	0.01	0.02	0.01
Controls	Mean	0.02	0.05	0.04	0.07
	SD	0.02	0.04	0.03	0.08

Lymphocytes ($X10^9/L$)

Group		Baseline	Week 4	Week 8	Week 12
8 mg/kg bodyweight	Mean	2.01	2.48	2.54	2.11
	SD	0.50	0.38	0.19	0.21
40 mg/kg bodyweight	Mean	1.96	2.10	2.51	2.55
	SD	0.93	0.48	0.49	0.95
200 mg/kg bodyweight	Mean	1.65	2.22	2.88	2.78
	SD	0.83	0.75	0.80	0.42
Controls	Mean	1.49	2.33	1.94	2.37
	SD	0.52	0.24	0.53	0.26

Monocytes ($X10^9/L$)

Group		Baseline	Week 4	Week 8	Week 12
8 mg/kg bodyweight	Mean	0.23	0.31	0.24	0.31
	SD	0.07	0.07	0.15	0.37
40 mg/kg bodyweight	Mean	0.19	0.26	0.26	0.25
	SD	0.13	0.09	0.02	0.08
200 mg/kg bodyweight	Mean	0.22	0.16	0.24	0.21
	SD	0.07	0.05	0.05	0.04
Controls	Mean	0.21	0.30	0.24	0.28
	SD	0.09	0.05	0.10	0.09

Platelets ($X10^9/L$)

Group		Baseline	Week 4	Week 8	Week 12
8 mg/kg bodyweight	Mean	288.50	259.50	254.75	246.50
	SD	58.86	11.45	33.15	24.37
40 mg/kg bodyweight	Mean	322.75	273.75	328.75	292.50
	SD	31.61	16.03	63.42	36.59
200 mg/kg bodyweight	Mean	339.50	288.75	339.00	303.75
	SD	10.15	24.27	35.75	31.84
Controls	Mean	287.50	240.50	263.75	253.25
	SD	32.42	30.03	29.61	45.12

Appendix III: Biochemistry (Graphs)

Appendix IV: Biochemistry (Means and standard deviations)

Calcium (mmol/L)

Group		Baseline	Week 4	Week 8	Week 12
8 mg/kg bodyweight	Mean	2.27	2.20	2.19	2.17
	SD	0.10	0.11	0.09	0.08
40 mg/kg bodyweight	Mean	2.20	2.15	2.16	2.13
	SD	0.04	0.06	0.04	0.03
200 mg/kg bodyweight	Mean	2.21	2.18	2.19	2.12
	SD	0.06	0.10	0.08	0.07
Controls	Mean	2.31	2.24	2.29	2.18
	SD	0.08	0.08	0.07	0.08

Magnesium (mmol/L)

Group		Baseline	Week 4	Week 8	Week 12
8 mg/kg bodyweight	Mean	0.66	0.62	0.63	0.57
	SD	0.04	0.03	0.06	0.02
40 mg/kg bodyweight	Mean	0.66	0.60	0.61	0.58
	SD	0.05	0.03	0.04	0.04
200 mg/kg bodyweight	Mean	0.62	0.62	0.61	0.57
	SD	0.06	0.03	0.02	0.02
Controls	Mean	0.65	0.63	0.62	0.58
	SD	0.04	0.02	0.02	0.04

Sodium (mmol/L)

Group		Baseline	Week 4	Week 8	Week 12
8 mg/kg bodyweight	Mean	144.75	146.25	146.50	144.75
	SD	1.50	1.50	1.29	0.96
40 mg/kg bodyweight	Mean	146.00	145.50	148.00	145.25
	SD	0.00	1.73	2.16	2.22
200 mg/kg bodyweight	Mean	145.25	146.25	147.25	145.25
	SD	0.96	1.26	1.89	0.96
Controls	Mean	146.00	148.25	149.25	146.50
	SD	2.16	2.75	2.87	2.89

Potassium (mmol/L)

Group		Baseline	Week 4	Week 8	Week 12
8 mg/kg bodyweight	Mean	3.38	3.18	3.43	3.23
	SD	0.30	0.10	0.25	0.13
40 mg/kg bodyweight	Mean	3.13	3.20	3.48	3.25
	SD	0.10	0.12	0.33	0.17
200 mg/kg bodyweight	Mean	3.10	3.23	3.48	3.18
	SD	0.22	0.25	0.13	0.22
Controls	Mean	3.20	3.03	3.45	3.10
	SD	0.16	0.15	0.06	0.14

Chloride (mmol/L)

Group		Baseline	Week 4	Week 8	Week 12
8 mg/kg bodyweight	Mean	105.25	108.75	110.75	109.00
	SD	0.96	0.96	1.89	1.41
40 mg/kg bodyweight	Mean	108.00	108.25	112.50	108.50
	SD	0.82	2.06	1.29	2.52
200 mg/kg bodyweight	Mean	107.00	108.00	110.50	107.50
	SD	2.16	1.63	2.08	1.29
Controls	Mean	107.50	110.00	111.75	110.50
	SD	3.11	2.45	2.06	3.11

Phosphate (mmol/L)

Group		Baseline	Week 4	Week 8	Week 12
8 mg/kg bodyweight	Mean	1.00	1.03	1.12	1.09
	SD	0.09	0.23	0.23	0.09
40 mg/kg bodyweight	Mean	0.87	0.91	1.15	1.00
	SD	0.10	0.07	0.11	0.19
200 mg/kg bodyweight	Mean	0.84	0.76	0.91	0.79
	SD	0.13	0.24	0.13	0.07
Controls	Mean	1.06	0.92	1.14	0.78
	SD	0.33	0.22	0.38	0.32

Albumin (g/L)

Group		Baseline	Week 4	Week 8	Week 12
8 mg/kg bodyweight	Mean	43.85	42.40	40.13	41.35
	SD	3.65	2.60	2.91	3.67
40 mg/kg bodyweight	Mean	44.18	41.15	39.40	40.88
	SD	2.29	3.18	2.79	1.92
200 mg/kg bodyweight	Mean	42.83	41.98	39.98	38.70
	SD	2.13	1.74	2.75	4.11
Controls	Mean	47.30	46.63	44.03	40.88
	SD	4.52	6.83	4.28	5.84

Globulin (g/L)

Group		Baseline	Week 4	Week 8	Week 12
8 mg/kg bodyweight	Mean	26.38	18.68	18.43	17.65
	SD	2.63	1.25	0.62	1.05
40 mg/kg bodyweight	Mean	20.60	18.05	19.18	18.93
	SD	2.95	3.31	3.48	3.17
200 mg/kg bodyweight	Mean	21.30	16.55	18.23	16.73
	SD	2.34	1.85	0.67	1.68
Controls	Mean	20.38	14.75	18.48	18.38
	SD	4.87	4.23	2.76	3.03

Total Proteins (g/L)

Group		Baseline	Week 4	Week 8	Week 12
8 mg/kg bodyweight	Mean	70.23	61.08	58.53	59.00
	SD	1.13	1.64	2.38	3.35
40 mg/kg bodyweight	Mean	64.78	59.20	58.60	59.80
	SD	4.04	1.15	1.92	1.91
200 mg/kg bodyweight	Mean	64.13	58.53	58.25	55.43
	SD	2.48	1.35	2.15	2.63
Controls	Mean	67.68	61.38	62.50	59.25
	SD	4.98	3.05	4.57	3.97

Creatine Kinase (u/L)

Group		Baseline	Week 4	Week 8	Week 12
8 mg/kg bodyweight	Mean	652.50	451.00	476.25	509.00
	SD	370.27	255.04	295.64	129.04
40 mg/kg bodyweight	Mean	1528.00	247.00	577.00	405.00
	SD	2002.97	212.14	97.64	149.29
200 mg/kg bodyweight	Mean	568.25	390.50	322.75	541.50
	SD	185.34	152.34	229.36	308.40
Controls	Mean	558.25	530.50	723.50	313.75
	SD	263.45	158.50	409.88	148.54

Lactate Dehydrogenase (u/L)

Group		Baseline	Week 4	Week 8	Week 12
8 mg/kg bodyweight	Mean	1508.75	1517.50	1158.00	1101.75
	SD	326.12	303.13	125.92	139.05
40 mg/kg bodyweight	Mean	1345.50	1467.75	1205.25	1273.75
	SD	384.36	195.16	221.51	184.98
200 mg/kg bodyweight	Mean	1151.50	1306.00	1125.00	1316.25
	SD	229.25	273.37	392.80	384.95
Controls	Mean	1047.50	1379.75	1060.50	1186.25
	SD	234.77	176.62	246.93	180.71

Gamma Glutamyl Transferase (u/L)

Group		Baseline	Week 4	Week 8	Week 12
8 mg/kg bodyweight	Mean	160.00	113.50	90.75	85.50
	SD	103.73	70.44	49.20	27.09
40 mg/kg bodyweight	Mean	94.75	77.50	69.75	82.50
	SD	37.16	30.16	24.14	32.09
200 mg/kg bodyweight	Mean	83.25	107.00	78.75	90.25
	SD	20.17	42.18	26.08	28.83
Controls	Mean	121.75	82.75	62.00	67.75
	SD	47.07	32.71	19.10	25.62

Alanine Aminotransferase (u/L)

Group		Baseline	Week 4	Week 8	Week 12
8 mg/kg bodyweight	Mean	27.50	52.50	75.75	75.00
	SD	12.01	12.01	50.91	36.90
40 mg/kg bodyweight	Mean	30.00	68.75	72.00	137.50
	SD	10.80	42.07	32.63	52.85
200 mg/kg bodyweight	Mean	41.00	104.25	105.50	121.75
	SD	20.46	108.69	80.33	87.85
Controls	Mean	26.50	29.25	31.00	41.75
	SD	17.90	10.11	4.32	14.41

Aspartate Aminotransferase (u/L)

Group		Baseline	Week 4	Week 8	Week 12
8 mg/kg bodyweight	Mean	166.50	56.25	70.75	63.00
	SD	49.06	14.66	18.10	30.14
40 mg/kg bodyweight	Mean	111.75	60.00	62.00	119.00
	SD	51.02	42.52	22.38	73.67
200 mg/kg bodyweight	Mean	128.00	115.00	69.75	144.75
	SD	21.32	138.65	26.30	191.00
Controls	Mean	73.00	36.50	47.25	48.50
	SD	20.05	5.80	9.07	41.23

Alkaline Phosphatase (u/L)

Group		Baseline	Week 4	Week 8	Week 12
8 mg/kg bodyweight	Mean	189.25	197.75	176.00	143.00
	SD	59.85	80.18	61.74	43.73
40 mg/kg bodyweight	Mean	174.50	161.75	145.75	138.50
	SD	49.47	37.21	47.64	20.24
200 mg/kg bodyweight	Mean	199.50	211.50	184.50	149.75
	SD	35.49	56.10	60.03	42.17
Controls	Mean	253.25	241.75	239.50	169.25
	SD	162.66	118.18	105.01	92.09

Total Bilirubin (μmol/L)

Group		Baseline	Week 4	Week 8	Week 12
8 mg/kg bodyweight	Mean	3.13	3.55	3.63	3.55
	SD	0.88	1.61	1.80	1.47
40 mg/kg bodyweight	Mean	3.30	3.35	3.38	3.68
	SD	0.71	0.58	0.91	1.62
200 mg/kg bodyweight	Mean	3.05	3.70	3.43	3.63
	SD	0.31	0.58	0.78	0.78
Controls	Mean	3.85	4.00	4.03	4.05
	SD	1.56	1.87	1.91	1.81

Direct Bilirubin (μmol/L)

Group		Baseline	Week 4	Week 8	Week 12
8 mg/kg bodyweight	Mean	1.28	1.68	1.55	2.35
	SD	0.38	0.85	0.83	1.07
40 mg/kg bodyweight	Mean	1.45	1.63	1.43	2.28
	SD	0.38	0.28	0.51	1.22
200 mg/kg bodyweight	Mean	1.43	1.75	1.50	2.48
	SD	0.15	0.19	0.50	0.64
Controls	Mean	1.53	1.73	1.73	2.65
	SD	0.46	0.57	0.72	1.03

Glucose (mmol/L)

Group		Baseline	Week 4	Week 8	Week 12
8 mg/kg bodyweight	Mean	3.75	4.48	3.80	4.23
	SD	0.47	0.67	0.08	0.78
40 mg/kg bodyweight	Mean	4.75	4.75	5.20	4.63
	SD	0.57	0.76	1.07	0.93
200 mg/kg bodyweight	Mean	4.70	4.95	5.20	5.03
	SD	0.37	0.44	0.82	0.97
Controls	Mean	6.20	5.88	4.35	4.93
	SD	1.03	0.68	0.83	0.95

Urea (mmol/L)

Group		Baseline	Week 4	Week 8	Week 12
8 mg/kg bodyweight	Mean	2.70	2.88	2.68	2.38
	SD	1.39	0.79	1.03	0.46
40 mg/kg bodyweight	Mean	3.05	4.10	3.53	3.28
	SD	1.14	1.09	1.31	2.50
200 mg/kg bodyweight	Mean	5.18	3.43	3.60	3.43
	SD	1.71	0.85	0.42	0.72
Controls	Mean	4.10	3.25	4.00	2.35
	SD	1.31	1.09	0.80	1.08

Creatinine (μmol/L)

Group		Baseline	Week 4	Week 8	Week 12
8 mg/kg bodyweight	Mean	109.50	80.00	80.00	77.00
	SD	5.00	8.37	7.79	2.45
40 mg/kg bodyweight	Mean	110.00	80.00	88.00	77.25
	SD	8.16	9.02	5.89	9.39
200 mg/kg bodyweight	Mean	110.25	92.50	88.50	78.25
	SD	7.37	15.42	9.33	13.07
Controls	Mean	114.25	101.50	81.00	78.00
	SD	6.50	7.00	10.13	13.14

Total Cholesterol (mmol/L)

Group		Baseline	Week 4	Week 8	Week 12
8 mg/kg bodyweight	Mean	5.62	4.62	4.62	4.57
	SD	0.50	0.27	0.08	0.32
40 mg/kg bodyweight	Mean	4.78	4.57	4.73	4.59
	SD	1.18	0.79	1.17	0.68
200 mg/kg bodyweight	Mean	4.54	3.79	4.04	3.90
	SD	1.01	0.44	0.46	0.45
Controls	Mean	4.14	4.29	4.47	4.21
	SD	0.72	0.77	0.94	1.07

High Density Lipoprotein- Cholesterol (mmol/L)

Group		Baseline	Week 4	Week 8	Week 12
8 mg/kg bodyweight	Mean	2.06	2.19	2.11	2.10
	SD	0.49	0.26	0.22	0.22
40 mg/kg bodyweight	Mean	2.30	2.12	2.04	2.18
	SD	0.45	0.58	0.54	0.60
200 mg/kg bodyweight	Mean	2.20	2.11	2.11	2.05
	SD	0.41	0.38	0.35	0.37
Controls	Mean	2.35	2.14	2.12	2.00
	SD	0.58	0.68	0.73	0.79

Low Density Lipoprotein-Cholesterol (mmol/L)

Group		Baseline	Week 4	Week 8	Week 12
8 mg/kg bodyweight	Mean	3.56	2.43	2.51	2.47
	SD	0.73	0.51	0.30	0.35
40 mg/kg bodyweight	Mean	2.48	2.46	2.69	2.42
	SD	0.85	0.44	0.96	0.32
200 mg/kg bodyweight	Mean	2.35	1.68	1.94	1.85
	SD	0.83	0.27	0.46	0.24
Controls	Mean	1.79	2.15	2.35	2.21
	SD	0.33	0.33	0.22	0.33

Appendix V: Physical and physiological variables (Graphs)

Appendix VI: Physical and physiological variables (Means and standard deviations)

Bodyweight (kg)

Group		Baseline	Week 4	Week 8	Week 12
8 mg/kg bodyweight	Mean	3.64	3.53	3.65	3.50
	SD	0.18	0.15	0.14	0.12
40 mg/kg bodyweight	Mean	3.64	3.67	3.75	3.59
	SD	0.20	0.28	0.25	0.18
200 mg/kg bodyweight	Mean	3.44	3.47	3.64	3.51
	SD	0.31	0.38	0.37	0.39
Controls	Mean	2.99	3.10	3.11	3.06
	SD	0.26	0.29	0.32	0.31

Body Temperature (°C)

Group		Baseline	Week 4	Week 8	Week 12
8 mg/kg bodyweight	Mean	39.18	38.73	38.98	39.03
	SD	0.30	0.21	0.33	0.05
40 mg/kg bodyweight	Mean	38.93	38.80	39.05	38.95
	SD	0.17	0.27	0.17	0.29
200 mg/kg bodyweight	Mean	38.95	38.70	39.08	38.73
	SD	0.21	0.42	0.43	0.53
Controls	Mean	39.43	39.40	39.30	38.90
	SD	0.22	0.28	0.29	0.24

Systolic Pressure (mmHg)

Group		Baseline	Week 4	Week 8	Week 12
8 mg/kg bodyweight	Mean	101.00	94.25	97.50	95.50
	SD	12.19	6.65	9.11	9.33
40 mg/kg bodyweight	Mean	98.25	95.50	90.75	95.50
	SD	7.68	8.54	6.99	5.92
200 mg/kg bodyweight	Mean	96.00	92.75	94.50	88.25
	SD	14.09	8.38	6.86	10.18
Controls	Mean	81.25	88.25	84.25	84.00
	SD	4.50	7.76	10.21	11.69

Diastolic Pressure (mmHg)

Group		Baseline	Week 4	Week 8	Week 12
8 mg/kg bodyweight	Mean	49.75	47.75	45.25	43.75
	SD	14.17	9.07	7.09	10.72
40 mg/kg bodyweight	Mean	45.00	50.25	41.00	51.00
	SD	2.94	10.63	9.20	2.00
200 mg/kg bodyweight	Mean	40.25	42.75	41.25	42.75
	SD	8.42	7.04	2.22	3.20
Controls	Mean	39.50	41.25	41.25	41.75
	SD	7.94	7.50	8.06	4.50

Mean Arterial Pressure (mmHg)

Group		Baseline	Week 4	Week 8	Week 12
8 mg/kg bodyweight	Mean	69.50	64.75	64.00	59.75
	SD	18.48	11.35	11.20	10.34
40 mg/kg bodyweight	Mean	62.75	72.25	59.75	75.75
	SD	8.54	15.63	15.71	2.06
200 mg/kg bodyweight	Mean	58.50	61.75	60.00	60.50
	SD	15.02	9.00	2.45	4.04
Controls	Mean	55.75	56.50	55.25	57.50
	SD	11.53	9.75	8.42	6.45

Pulse (beats/min)

Group		Baseline	Week 4	Week 8	Week 12
8 mg/kg bodyweight	Mean	137.75	155.75	148.50	136.25
	SD	8.81	24.25	16.78	7.37
40 mg/kg bodyweight	Mean	136.25	133.75	136.50	140.75
	SD	5.85	10.31	8.58	12.58
200 mg/kg bodyweight	Mean	148.50	148.50	167.50	149.50
	SD	22.71	35.72	26.89	20.87
Controls	Mean	162.25	161.75	148.50	163.75
	SD	21.93	20.40	22.49	27.58

Respiratory rate (breaths/min)

Group		Baseline	Week 4	Week 8	Week 12
8 mg/kg bodyweight	Mean	27.00	24.00	24.00	28.50
	SD	3.46	4.90	4.90	5.74
40 mg/kg bodyweight	Mean	21.00	21.00	24.00	21.00
	SD	3.46	3.46	4.90	3.46
200 mg/kg bodyweight	Mean	24.00	27.00	30.00	21.00
	SD	4.90	3.46	4.90	3.46
Controls	Mean	31.50	24.00	24.00	24.00
	SD	5.74	4.90	8.49	4.90

Appendix VII: Checklists

Appendix VIII: Certificates of analyses