Vipragen

Study Report

Study Title	:	"Effect of Amrete Packaged Drinking water on stamina and fatigue in Swiss albino mice"
Study No.	:	VBPL-P010/18
Version	:	01
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I. STUDY REPORT AGREEMENT

Responsibility	Personnel/Email/Phone	Sign & date
Study Director	Dr. Kumaraswamy kumaraswamy.mv@vipragen.com +91-9611398328	24/07/2019
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II. SUMMARY

Fatigue is best defined as difficulty in initiating or sustaining voluntary activities. Fatigue is accompanied by a feeling of extreme physical or mental tiredness, resulting from severe stress and hard physical or mental work. Two mechanisms can explain the occurrence of physical fatigue: oxidative stress and energy exhaustion. Exhaustive or intensive exercise can lead to the accumulation of excess reactive free radicals, resulting in tissue damage. Exhaustion theory suggests that energy source depletion and excess metabolite accumulation can lead to fatigue. The forced swimming test in mice is a widely-used experimental method to evaluate anti-fatigue effects, in which the onset of fatigue and weariness and utilization/depletion of glycogen has been shown similar both in rodents and humans.

The present study investigates the anti-fatigue activity and potential to improve the strength and stamina of Amrete Packaged Drinking water in Swiss albino mice. Animals were divided into 5 groups, namely Non-swimming (G1), Swimming control (G2), Amrete 30 ppm (G3), 125 ppm (G4) and reference item, Revital-H (G5), comprising of 12 animals/group (6 male & 6 female). The following parameters were observed in the study; morbidity, mortality, clinical signs of toxicity, changes in body weight, food consumption, weekly swimming test and clinical pathology parameters.

The swimming exercise was employed to evaluate anti-fatigue activity of Amrete in mice as it is commonly accepted that swimming is an experimental exercise model (Lapveteläinen *et al.,* 1997). The treatment was carried out for a period of 28 days. There were no changes in body weight and feed consumption in Amrete treated groups compared to Non-swim and Swim control groups.

Swim Endurance test

Fatigue is a feeling of extreme tiredness which can result in a broad range of physical and mental unfitness including inattention, distraction, and drowsiness. It is mainly caused by the depletion of energy sources, which include the accumulation of end products of fatigue, the disorder of internal the environment, the decrease in glycemic levels and liver glycogen consumption. Fatigue is a kind of sub-health status and may be associated with many illnesses (Bao *et al.,* 2016).

In the present study, it was observed that the average swimming time of animals treated with Amrete 125 ppm increased with elevation of anti-fatigue activity after week 1, 2, 3 and 4 in comparison to vehicle control, indicating Amrete lengthened the swim endurance time. The observed effect was more pronounced at dosage of

Amrete 125 ppm than Amrete 30 ppm. The high dose of Amrete 125 ppm increased more than 50% in the swim endurance time compared to control in both males and female. The reference item, Revital-H showed 109.58 and 97.39% increase in the swim endurance time compared to control in males and females respectively.

Clinical pathology

Swimming exercise is known to induce clinical pathological changes. Swimming exercise induced clinical pathological changes were measured in order to determine and understand the anti-fatigue mechanism. Glycolysis of carbohydrates under anaerobic conditions is the main energy source for intense exercise in a short period of time and produces lactate as an end product. Therefore, lactate level is one of the important indicators for determining fatigue. Decrease in lactates after swimming can be used as an indicator for the degree of fatigue (Oh *et al.,* 2013). In the present study, lactate dehydrogenase (LDH) levels in the Amrete treated groups were found lesser than the control group indicating that Amrete could effectively increase the swim endurance time and postpone the onset of fatigue. There was no other treatment related changes in clinical chemistry parameters.

Creatinine kinase is an important clinical biomarker for muscle damage, such as muscular dystrophy, severe muscle breakdown, myocardial infarction, autoimmune myositides, and acute renal failure (Wu RE *et al.,2013*). In the present study, Amrete also ameliorate skeletal muscle injury induced by exercise challenge.

ATP level in muscles is another important biochemical parameter related to muscle fatigue. Aerobic metabolism is more efficient than anaerobic glycolysis in producing ATP which indicates antioxidants assist in the undisrupted oxygen supply that creates the aerobic environment. Besides inhibiting cell damage, antioxidants may enhance the overall physical performance under the stress conditions (Bao *et al.,* 2016). In the present study, Liver and muscle glycogen and muscle ATP levels increased in Amrete treated group in comparison to swimming control suggesting that Amrete treatment possibly resulted in anti-fatigue effects on mice.

Blood gases Analysis

Swim exercise in animals, triggers the respiratory compensatory mechanisms. The relative excess of carbonic acid and the accumulation of volatile acid products irritate directly the peripheral and central haemo receptors of the respiratory center through a nervous reflector way, thus resulting in a compensatory hyperventilation. This is in accordance with the decrease in pCO_2 (Goundasheva, 2000). In the current study, the

level of pO_2 of increased in the Amrete treated group compared to swim control in females. All other parameters biochemical and blood gases showed similar to vehicle control group.

CONCLUSIONS

The study shows that daily treatment of Amrete in male and female Swiss mice caused

1. No mortalities and clinical signs of toxicity
2. No changes in body weight and food consumption
3. Increased Anti-fatigue activity in both male and female
4. Decreased lactate dehydrogenase (LDH) and creatinine kinase (CK) in males.
5. Increased muscle glycogen, liver glycogen and muscle ATP levels in both male and female

Based on these observations, it is concluded that daily administration of Amrete Packaged drinking water at the dose levels of 30 and 125 ppm for 28- days increased Anti-fatigue activity in Swiss mice. It increased muscle glycogen, liver glycogen, muscle ATP levels and decreased lactate dehydrogenase (LDH) and creatinine kinase levels in both sexes. Amrete at the highest tested dose of 125 ppm showed significant increase in the swim endurance time and Anti-fatigue activity in males. Amrete showed good results in females also but the increase in anti-fatigue activity was less than males.

III. LIST OF ABBREVIATIONS

ATP	Adenosine triphosphate
CPCSEA	Committee for the Purpose of Control and Supervision of Experiments on Animals
°C	Degree Celsius
CK	Creatinine kinase
CRE	Creatinine
DDW	Deuterium –depleted water
Hbg	Hemoglobin
hrs	Hour/s
kg	Kilogram/s
mg	Milligram/s
mL	Milliliter
PCO_2	Partial pressure of carbon dioxide
pH	Power of hydrogen
p.o	Per oral
PO_2	Partial pressure of oxygen
RI	Reference item
RH	Relative humidity
TI	Test item

IV. STUDY INFORMATION

Study Title	Effect of Amrete Packaged Drinking water on stamina and fatigue in Swiss albino mice.
Study Number	VBPL-P010/18
Test Item Name.	Amrete Packaged Drinking water (Deuterium-depleted water)
Study Director	Dr. Kumaraswamy.MV
Test Facility	Vipragen Biosciences Pvt. Ltd. #67b, Hootagalli industrial area, Mysore 570 018, Karnataka, India. Phone +91-821-4194611
Sponsor	M/s. Amrete Healthcare (India) P Ltd. 17, Lotus Business Park, New-Link Road, Andheri - West Mumbai - 400053
Sponsor Nominee	Mr. Manish Kothari manish.k@clearsynth.com +91- 9820926635

V. STUDY SCHEDULE

Description of activity	Dates of activity
Study initiation Date	22 October 2018
Experiment start date	24 October 2018
Necropsy/Experiment completion	21 November 2018
Draft report	19 February 2019
Final report	24 July 2019

VI. LIST OF STUDY PERSONNEL

Name	Responsibility
Dr. Arpana R. Sontakke	Veterinarian
Mr. Pradeep R	Study personnel
Mr. Rajath KK	Study personnel
Mr. Paramesh A J	Animal Technician

1. STUDY OBJECTIVE

The objective of this study was to evaluate the effect of Amrete Packaged Drinking water (Deuterium-depleted water) on stamina and fatigue in Swiss albino mice using swim endurance test.

Principle of the study:

Fatigue is best defined as difficulty in initiating or sustaining voluntary activities. Fatigue is accompanied by a feeling of extreme physical or mental tiredness, resulting from severe stress and hard physical or mental work. Two mechanisms can explain the occurrence of physical fatigue: oxidative stress and energy exhaustion. Exhaustive or intensive exercise can lead to the accumulation of excess reactive free radicals, resulting in tissue damage. Exhaustion theory suggests that energy source depletion and excess metabolite accumulation can lead to fatigue. The forced swimming test in mice is widely-used experimental method to evaluate anti-fatigue treatments in which the onset of fatigue and weariness, utilization/depletion of glycogen has been shown similar in rodents and humans.
Swim endurance test is commonly used to evaluate the efficacy of products against fatigue using Swiss albino mice.

2. ANIMAL WELFARE

This protocol was approved by the Institutional Animal Ethics Committee (IAEC) vide protocol number VIP/IAEC/135/2018. The experiments were conducted as per the recommendation of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines on the regulation of scientific experiments on animals, Ministry of Environment & Forests (Animal Welfare Division) Government of India, June 2007.

3. MATERIALS AND METHODS

3.1. TEST ITEM DETAILS

The test item information as furnished by the sponsor is as follows

TI name	:	Amrete Packaged Drinking water
Strength	:	125ppm ± 5ppm and 30ppm ± 5ppm
Sample description	:	DDW water, HSN/SAC Code-00440249

Physical state	:	Liquid
Storage conditions	:	RT

Amrete Packaged Drinking Water (Low in Deuterium – 125ppm ± 5ppm and 30ppm ± 5ppm).

Added Salts & Minerals - (typically 100ml contains)

Magnesium sulphate Eq. to 0.2mg of magnesium, Potassium Bicarbonate Eq. to 0.3mg of Potassium, Calcium chloride eq. to 0.7mg of calcium

Note: The identity and composition of the test item is the responsibility of the sponsor.

3.2. REFERENCE ITEM DETAILS

The reference item information as furnished is as follows:

RI name	:	Revital-H
Manufacturer	:	Sun Pharma laboratories ltd.
Physical state	:	Capsule
Storage conditions	:	RT

3.3. FORMULATION PREPARATION

Test item: Since test item is packaged Deuterium-depleted drinking water and the same was provided in liquid form if necessary the doses were adjusted according to sponsor suggestions.

Reference item: Reference item Revital-H was suspended in Milli-Q water into a final concentration of 200 mg/ml.

3.4. TEST SYSTEM DETAILS

Animal species	Mice
Strain	Swiss Albino
Sex	Male and Female
Source	Animals procured from any CPCSEA registered Animal provider
Duration of the Study	28 days

Number of groups	5
No. of animals per group	6 Male + 6 Female
Total number of animals	60
Age at treatment	8-10 weeks
Body weight at treatment	Male 25±5g, Female 23±5 g
Identification	Animals were identified by tail marking using permanent animal marker. The individual animal number was recorded and maintained along with the raw data sheet/worksheet. Cage card was placed on each individual cage with details such as study no, study code, animal no, test item name, group, dose, species, sex.

3.5. ANIMAL HUSBANDRY

The following husbandry conditions were maintained during the study period.

a) Conditions: Animals were housed under standard laboratory conditions, air-conditioned with adequate fresh air supply (10-15 Air changes per hour), RT 22 ± 3°C, RH 30-70 %, with 12 hours light and 12 hours dark cycle in sequence. The temperature and RH were recorded once daily.

b) Housing: Animals were housed up to 3 per cage in standard polypropylene (Size: L 320 x B 170 x H 140 mm) having facilities for holding pellet feed and drinking water in water bottle fitted with stainless steel sipper tube.

d) Diet: The animals were fed *ad libitum* with normal rodent feed throughout the study period.

e) Water: Reverse osmosis water were provided *ad libitum* throughout the acclimatization to all animals and during treatment period, for G1, G2 and G5. Water was provided in water bottles with stainless steel sipper tubes.

d) Bedding material: Autoclaved corn cob was used as bedding material for the animal cages. The bedding material was changed routinely as per the standards.

3.6. GROUPING AND STUDY DESIGN

Grouping of animals were done by using Swim Endurance time and ensured that body weight doesn't exceed ± 20% of the mean body weight within the group. A total of 30 males and 30 female Swiss Albino mice aged 8-10 weeks free from diseases with good health status were chosen for the study. The animals were grouped into 5 groups as presented in Table 1. The animals from G1 group not received any treatment and considered as placebo treatment group, G2 was swimming control with vehicle, G3 and G4 animals were treated with Amrete at 30 ppm and 125 ppm in water bottles with stainless steel sipper tubes. G5 was dosed with Reference item Revital at 200 mg/kg bw per oral (p.o).

Table 1: Group details of the study

Groups	Dose Groups	Doses	Route	Duration (in days)	No. of animals/group
1	Non Swimming control	-	Administered as drinking water	28	6M + 6F
2	Swimming control	-			6M + 6F
3	Amrete + Swimming	30 ppm			6M + 6F
4	Amrete + Swimming	125 ppm			6M + 6F
5	Reference item (Revital-H) + Swimming	200 (mg/kg)	Oral (gavage)		6M + 6F
Total no. of animals					**60**

3.7. DOSE SELCTION

The dose levels of Amrete were selected as suggested by sponsor.

3.8. ROUTE OF ADMINISTATION

Amrete was provided by bottles with stainless steel sipper tubes. Oral is the intended route of administration in humans.

3.9. DOSE VOLUME AND FREQUENCY OF ADMINISTRATION

Amrete was provided as drinking water through stainless steel sipper tubes. Animals from control received normal water and from G3-G4 received Amrete drinking water at 30 and 125 ppm, respectively. Revital-H was administered through oral (gavage). Animals received vehicle or TI or RI for a period of 4 weeks.

3.10. IN-LIFE OBSERVATIONS AND MEASUREMENTS

3.10.1. Morbidity and mortality

Animals were observed twice daily for morbidity and mortality during the entire duration of study period.

3.10.2. Clinical signs of toxicity

All the animals were observed for test item treatment related clinical signs of toxicity. The animals were observed for adverse effect on respiratory, cardiovascular, gastrointestinal and nervous system. Animals with severe clinical signs or distress were humanely sacrificed and were recorded in the raw data.

3.10.3. Swim endurance test

The swimming exercise of mice was measured with a plastic round tub (62 × 28 cm) filled with water maintained at 34±1°C. All the animals were allowed to swim till exhaustion. The end point of Swim Endurance (SE) test was considered when the mouse drowned more than thrice. SE was performed on Day 0, 7, 14, 21 and 28; Percent anti fatigue activity was calculated using formula:

$$\% \text{ Anti fatigue Activity} = \frac{(\text{TI groups SE time} - \text{Control groups SE time})}{\text{Control SE time}} \times 100$$

3.10.4. Body weight

All the animals' body weight was recorded once in a week during treatment.

3.10.5. Feed consumption

Quantity of feed consumption was measured once in a week. The amount of feed offered and left over was recorded for each cage and feed intake (g/mice/day) was calculated.

3.10.6. TI consumption

Amrete was provided as liquid through stainless steel sipper tubes and daily offered and left over was recorded for each cage and calculated (mL/day/cage).

3.10.7. Clinical pathology

Blood samples were collected immediately after the swim endurance test on day 28 of the study. Blood sample was collected by heart puncture under isoflurane-oxygen anesthesia.

Clinical Chemistry

The following clinical chemistry parameters were estimated at the end of study period.

Parameters	Units
Lactate	mmol/L
Hemoglobin	g/dL
Urea	mg/dL
CRE	mg/dL
Lactate dehydrogenase	U/L
Creatinine kinase	U/L
pH	-
PCo2	mmHg
Po2	mmHg
$Chco_3$	mmol/L
BE(ecf)	mmol/L
Na+	mmol/L
K+	mmol/L
Ca++	mmol/L
Cl-	mmol/L
$Ctco_2$	mmol/L
Hgb	g/dl

3.10.8. Tissue ATP and Glycogen estimation

Liver and muscle were collected, flash frozen in liquid nitrogen and stored in -80˚C until analysis. Glycogen levels were estimated from liver and muscle tissue and ATPs were measured in muscle tissue.

4. NECROPSY

4.1. UNSCHEDULED DEATHS

No unscheduled deaths were observed during study period.

4.2. SCHEDULED EUTHANASIA

All the animals were euthanized using over-dose of isoflurane-oxygen. These animals were undergone necropsy and specified tissues were retained.

4.3. GROSS NECROPSY

All animals in the study were subjected to a full, detailed gross necropsy which includes careful examination of the external surface of the body, all orifices, the cranial, thoracic and abdominal cavities and their contents.

5. STATISTICAL ANALYSIS

The following statistical methods were used to analyze the body weight and feed consumption as well as clinical pathology data after treatment

- Data was summarized in tabular form. Statistical analysis was performed using Graph Pad Prism 7.04 program.
- All the data was checked for normality with Shapiro-Wilk
- Data for each group of animals was subjected to analysis of variance (ANOVA). Values was given as mean ±standard deviation (SD)
- All analyses and comparisons were evaluated at the 5% ($P \leq 0.05$) level.

6. RESULTS

6.1.1. Morbidity and mortality

There were no moralities observed during the study in any of the treated and control groups.

Group wise summary data are presented in Table 1. Individual animal data are presented in Appendix 1&2.

6.1.2. Body weight

There was no treatment related body weight change observed during the study.

Group wise summary data are presented in Table 2&3 and Figure 1&2. Individual animal data are presented in Appendix 3&4.

6.1.3. Amrete/vehicle consumption

There was no treatment related change observed in daily Amrete/vehicle consumption.

Group wise summary data are presented in Table 4&5. Individual animal data are presented in Appendix 5.

6.1.4. Feed consumption

There was no treatment related change observed in weekly feed consumption in Amrete/vehicle groups.

Group wise summary data are presented in Table 6&7. Individual animal data are presented in Appendix 6.

6.1.5. Swim endurance test

The swim endurance time in males increased in Amrete 125 ppm from week 1 onwards and Amrete 30 ppm showed from week 3 onwards. The swim endurance time in females increased in Amrete 125 ppm from week 2 onwards and Amrete 30 ppm at 4^{th} week. The high dose of Amrete 125 ppm increased more than 50% in the swim endurance time compared to control in both males and females. The reference item, Revital-H showed 110 and 98% increase in the swim endurance time compared to control in males and females, respectively.

Group wise summary and individual animals' data all swim endurance time, % Anti-fatigue values are presented in Table 8-11 and Figure 3&4. Individual animal data are presented in Appendix 7&8.

6.1.6. Clinical pathology

Lactate dehydrogenase (LDH), creatinine kinase levels in the Amrete 125ppm treated groups were found lesser than the swim control group in males and no significant changes observed in females. There was no other treatment related changes in clinical chemistry parameters.

Group wise summary and individual animals' data all clinical chemistry parameters are presented in Table 12 & 13 and Figure 5&6 for male and female respectively. Individual animal data are presented in Appendix 9&10.

6.1.7. Tissue ATP and Glycogen estimation

Liver and muscle Glycogen levels

The level of glycogen in Liver and muscles were increased in the Amrete treated groups compared to control in both sexes.

Muscle ATP levels

The level of ATP was increased in the muscle of Amrete treated groups compared to control in both sexes.

Group wise summary and individual animals' data all liver, muscle glycogen levels and muscle ATP levels are presented in Table 14, Figure 7 and Appendix 11.

6.1.8. Blood gases Analysis

In the current study, the level of pO2 of increased in the Amrete 125 ppm treated female group compared to swim control. There was no other treatment related changes in blood gases parameters.

Group wise summary and individual animals' data all clinical chemistry parameters are presented in Table 12 & 13 and 5&6 for male and female respectively.

7. DISCUSSION

The present study investigates the anti-fatigue activity and potential to improve the strength and stamina of Amrete Packaged Drinking water in Swiss albino mice.

Animals were divided into 5 groups, namely Non-swimming (G1), Swimming control (G2), Amrete 30 ppm (G3), 125 ppm (G4) and reference item, Revital-H (G5), comprising of 12 animals/group (6 male & 6 female). The following parameters were observed in the study; morbidity, mortality, clinical signs of toxicity, changes in body weight, food consumption, weekly swimming test and clinical pathology parameters.

The swimming exercise was employed to evaluate anti-fatigue activity of Amrete in mice as it is commonly accepted that swimming is an experimental exercise model (Lapveteläinen et al., 1997). The treatment was carried out for a period of 28 days. There were no changes in body weight and feed consumption in Amrete treated groups compared to Non-swim and Swim control groups.

Swim Endurance test

Fatigue is a feeling of extreme tiredness which can result in a broad range of physical and mental unfitness including inattention, distraction, and drowsiness. It is mainly caused by the depletion of energy sources, which include the accumulation of end products of fatigue, the disorder of internal the environment, the decrease in glycemic levels and liver glycogen consumption. Fatigue is a kind of sub-health status and may be associated with many illnesses (Bao et al., 2016).

In the present study, it was observed that the average swimming time of animals treated with Amrete 125 ppm increased with elevation of anti-fatigue activity after week 1, 2, 3 and 4 in comparison to vehicle control, indicating Amrete lengthened the swim endurance time. The observed effect was more pronounced at dosage of Amrete 125 ppm than Amrete 30 ppm. The high dose of Amrete 125 ppm increased more than 50% in the swim endurance time compared to control in both males and female. The reference item, Revital-H showed 109.58 and 97.39% increase in the swim endurance time compared to control in males and females respectively.

Clinical pathology

Swimming exercise is known to induce clinical pathological changes. Swimming exercise induced clinical pathological changes were measured in order to determine and understand the anti-fatigue mechanism. Glycolysis of carbohydrates under anaerobic conditions is the main energy source for intense exercise in a short period of time and produces lactate as an end product. Therefore, lactate level is one of the important indicators for determining fatigue. Decrease in lactates after swimming can

be used as an indicator for the degree of fatigue (Oh et al., 2013). In the present study, lactate dehydrogenase (LDH) levels in the Amrete treated groups were found lesser than the control group indicating that Amrete could effectively increase the swim endurance time and postpone the onset of fatigue. There was no other treatment related changes in clinical chemistry parameters.

Creatinine kinase is an important clinical biomarker for muscle damage, such as muscular dystrophy, severe muscle breakdown, myocardial infarction, autoimmune myositides, and acute renal failure (Wu RE et al.,2013). In the present study, Amrete also ameliorate skeletal muscle injury induced by exercise challenge.

ATP level in muscles is another important biochemical parameter related to muscle fatigue. Aerobic metabolism is more efficient than anaerobic glycolysis in producing ATP which indicates antioxidants assist in the undisrupted oxygen supply that creates the aerobic environment. Besides inhibiting cell damage, antioxidants may enhance the overall physical performance under the stress conditions (Bao et al., 2016). In the present study, Liver and muscle glycogen and muscle ATP levels increased in Amrete treated group in comparison to swimming control suggesting that Amrete treatment possibly resulted in anti-fatigue effects on mice.

Blood gases Analysis

Swim exercise in animals, triggers the respiratory compensatory mechanisms. The relative excess of carbonic acid and the accumulation of volatile acid products irritate directly the peripheral and central haemo receptors of the respiratory center through a nervous reflector way, thus resulting in a compensatory hyperventilation. This is in accordance with the decrease in pCO2 (Goundasheva, 2000). In the current study, the level of pO2 of increased in the Amrete treated group compared to swim control in females. All other parameters biochemical and blood gases showed similar to vehicle control group.

CONCLUSIONS

The study shows that daily treatment of Amrete in male and female Swiss mice caused

1. No mortalities and clinical signs of toxicity
2. No changes in body weight and food consumption
3. Increased Anti-fatigue activity in both male and female

4. Decreased lactate dehydrogenase (LDH) and creatinine kinase (CK) in males.
5. Increased muscle glycogen, liver glycogen and muscle ATP levels in both male and female

Based on these observations, it is concluded that daily administration of Amrete Packaged drinking water at the dose levels of 30 and 125 ppm for 28- days increased Anti-fatigue activity in Swiss mice. It increased muscle glycogen, liver glycogen, muscle ATP levels and decreased lactate dehydrogenase (LDH) and creatinine kinase levels in both sexes. Amrete at the highest tested dose of 125 ppm showed significant increase in the swim endurance time and Anti-fatigue activity in males. Amrete showed good results in females also but the increase in anti-fatigue activity was less than males.

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8. TABLES

TABLE 1 - SUMMARY OF CLINICAL SIGNS AND MORTALITY

Groups	Doses	Mortality	Clinical Signs
G1-Non swimming control	0	0/12	NAD
G2-Swimming control	0	0/12	NAD
G3-Amrete + Swimming	30 ppm	0/12	NAD
G4-Amrete 125 ppm + Swimming	125 ppm	0/12	NAD
G5-Revital-H + Swimming	200 (mg/kg)	0/12	NAD

n=12 (6M and 6F); NAD – No Abnormalities Detected; values are incidence of mortality/total no. of animals.

TABLE 2 - SUMMARY OF BODY WEIGHTS OF MALE MICE

Groups & Doses	Week 0 (g)	Week 1 (g)	Week 2 (g)	Week 3 (g)	Week 4 (g)
G1-Non swimming control	25.55 ±4.13	26.66 ±5.69	26.33 ±5.24	26.15 ±5.28	26.74 ±5.80
G2-Swimming control	22.78 ±3.81	22.76 ±4.68	23.27 ±4.01	22.14 ±4.86	22.15 ±4.30
G3-Amrete 30 ppm + Swimming	24.15 ±2.17	24.30 ±2.29	23.16 ±2.65	22.94 ±3.03	23.17 ±3.12
G4-Amrete 125 ppm + Swimming	24.91 ±3.37	23.91 ±3.97	22.87 ±3.50	21.33 ±3.64	21.72 ±4.65
G5-Revital-H + Swimming	23.17 ±5.36	21.62 ±5.65	22.27 ±5.92	23.19 ±5.20	23.62 ±6.06

n=6; Values are in Mean±SEM ; $p<0.05$, * – significance with respect to G1, # – significance with respect to G2.

TABLE 3 – SUMMARY OF BODY WEIGHTS OF FEMALE MICE

Groups & Doses	Week 0 (g)	Week 1 (g)	Week 2 (g)	Week 3 (g)	Week 4 (g)
G1-Non swimming control	22.14 ±2.45	22.16 ±3.75	22.46 ±4.95	21.79 ±5.83	22.08 ±5.85
G2-Swimming control	23.66 ±4.71	23.35 ±5.65	23.7 ±5.53	23.04 ±5.92	23.88 ±4.56
G3-Amrete 30 ppm + Swimming	22.23 ±2.04	22.56 ±2.83	22.11 ±3.04	21.63 ±4.69	23.54 ±3.43
G4-Amrete 125 ppm + Swimming	22.77 ±5.80	24.15 ±5.09	24.03 ±5.55	23.36 ±5.12	23.53 ±4.68
G5-Revital-H + Swimming	19.72 ±0.97	19.76 ±1.89	19.76 ±1.72	19.79 ±3.01	20.59 ±2.25

n=6; Values are in Mean±SEM ; $p<0.05$, * – significance with respect to G1, # – significance with respect to G2.

TABLE 4 – SUMMARY OF DAILY AVERAGE AMRETE/VEHICLE CONSUMPTION OF MALE MICE

Groups & Doses	Days 1	2	3	4	5	6	7	8	9	10	11	12	13	14
G1-Non swimming control	4.65 ±0.41	3.72 ±0.33#	4.04 ±0.53	3.94 ±0.27	5.46 ±1.16	4.69 ±1.46	5.13 ±1.14	5.1 ±1.71	4.74 ±0.42	5.27 ±0.95	5.95 ±3.27	4.18 ±0.64	7.7 ±0.64	8.09 ±0.32
G2-Swimming control	3.96 ±0.06	2.89 ±0.2*	3.2 ±0.88	4.29 ±0.77	4.5 ±0.84	4.13 ±1.56	3.82 ±2.04	3.83 ±2.4	3.04 ±2.6	4.61 ±0.11	5.29 ±0.44	5.86 ±1.54	6.16 ±1.96	9.64 ±0.69
G3-Amrete 30 ppm + Swimming	4.19 ±0.22	3.23 ±0.17*	3.65 ±0.35	4.56 ±0.76	5.64 ±1.28	4.37 ±0.92	4.14 ±0.14	5.22 ±1.27	3.99 ±0.78	4.54 ±0.76	5.43 ±0.45	4.13 ±2.1	5.16 ±0.84*	7.46 ±0.17
G4-Amrete 125 ppm + Swimming	5.59 ±0.82#	3.88 ±0.16#	4.46 ±0.33#	4.64 ±0.49	4.72 ±0.35	3.89 ±0.17	4.29 ±0.54	4.87 ±0.31	4.13 ±0.25	4.00 ±0.03	5.32 ±0.26	5.21 ±0.48	6.19 ±0.2	6.8 ±0.15
G5-Revital-H + Swimming	5.3 ±1.78	2.91 ±0.43*	3.97 ±1.32	4.53 ±1.47	5.18 ±1.69	3.06 ±1.27	3.55 ±1.87	4.27 ±1.31	3.29 ±0.63	4.42 ±2.03	3.61 ±0.76	3.5 ±0.47	5.9 ±0.65*	8.25 ±1

n=6; Values are in Mean±SEM ; p<0.05, * – significance with respect to G1, # – significance with respect to G2.

TABLE 4 – CONTD., SUMMARY OF DAILY AVERAGE AMRETE/VEHICLE CONSUMPTION OF MALE MICE

Groups & Doses	Days 15	16	17	18	19	20	21	22	23	24	25	26	27
G1-Non swimming control	5.80 ±0.44	8.59 ±0.09	7.05 ±1.08	7.82 ±0.16	6.4 ±0.06#	5.37 ±0.42	6.43 ±0.5	6.07 ±0.67#	5.11 ±0.42	5.47 ±0.47#	7.19 ±0.42#	6.33 ±0.77#	5.24 ±0.34#
G2-Swimming control	6.22 ±2.38	9.69 ±1.51	6.95 ±1.16	8.07 ±1.15	3.9 ±1.56*	4.01 ±1.91	5.34 ±0.45	3.54 ±1.63*	3.74 ±0.23*#	3.37 ±0.3*	5.4 ±0.41*	3.69 ±0.86*	3.99 ±0.29*
G3-Amrete 30 ppm + Swimming	7.21 ±0.04	7.81 ±0.06	6.7 ±0.81	8.82 ±0.65	6.68 ±0.75#	6.12 ±1.19#	5.46 ±0.16	5.02 ±0.12	4.88 ±0.21	5.11 ±0.27#	7.71 ±0.6#	3.97 ±0.95*	3.96 ±1.17*
G4-Amrete 125 ppm + Swimming	6.81 ±0.13	8.27 ±0.71	8.06 ±0.73	6.93 ±0.15	5.46 ±0.5#	5.85 ±1.36	10.37 ±6.6#	6.23 ±0.85#	4.64 ±0.32	4.46 ±0.59*#	7.05 ±0.21#	3.89 ±0.09*	3.31 ±0.07*
G5-Revital-H + Swimming	6.97 ±0.11	8.99 ±0.6	8.67 ±1.02	6.94 ±1.43	5.34 ±0.49#	5.15 ±0.9	5.9 ±0.97	5.7 ±1.12#	4.2 ±0.48*#	4.17 ±0.44*#	6.43 ±0.07*#	5.54 ±0.89#	4.04 ±0.65*

n=6; Values are in Mean±SEM ; p<0.05, * – significance with respect to G1, # – significance with respect to G2.

TABLE 5 – SUMMARY OF DAILY AVERAGE AMRETE/VEHICLE CONSUMPTION OF FEMALE MICE

	Days													
Groups & Doses	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
G1-Non swimming control	3.13 ±0.59	2.37 ±0.7	3.59 ±0.68	3.7 ±0.68	3.17 ±0.37	3.44 ±0.7	3.61 ±0.55	3.51 ±0.84	2.71 ±0.19	4.45 ±0.05	4.76 ±0.64	7.44 ±1.43	8.51 ±1.2#	9.26 ±0.02
G2-Swimming control	4.19 ±0.04	3.32 ±0.05	3.67 ±0.34	3.35 ±0.29	4.00 ±0.85	3.79 ±0.32	3.19 ±0.00	3.71 ±0.23	3.92 ±0.19	3.95 ±0.99	5.89 ±1.25	8.58 ±1.15	10.71 ±0.43*	9.10 ±0.21
G3-Amrete 30 ppm + Swimming	3.23 ±0.72	1.4 ±0.16	2.59 ±0.33	4.05 ±0.18	3.31 ±0.26	3.75 ±0.33	3.79 ±0.76	3.5 ±0.99	2.81 ±1.07	4.44 ±0.69	4.23 ±0.35	7.55 ±0.64	8.55 ±2#	7.63± 1.45
G4-Amrete 125 ppm + Swimming	3.9 ±0.13	3.94 ±3.51	5.51 ±3.36	5.47 ±2.38#	5.57 ±1.94*	6.34 ±2.27*#	6.36 ±1.68*#	5.52 ±1.4	4.79 ±2.25	6.85 ±0.69*#	4.76 ±2.03	6.97 ±0.25#	7.44 ±1.18#	8.12 ±0.74
G5-Revital-H + Swimming	1.73 ±0.18	2.19 ±0.36	3.29 ±0.2	2.78 ±1.04	2.34 ±0.27	2.91 ±0.04	3.47 ±0.2	2.69 ±0.28	2.45 ±0.63	3.79 ±0.42	3.93 ±0.87#	6.32 ±0.05#	7.36 ±0.8#	7.29 ±0.21

n=6; Values are in Mean±SEM ; p<0.05, * – significance with respect to G1, # – significance with respect to G2.

TABLE 5 – CONTD., SUMMARY OF DAILY AVERAGE AMRETE/VEHICLE CONSUMPTION OF FEMALE MICE

	Days												
Groups & Doses	**Day 15**	**Day 16**	**Day 17**	**Day 18**	**Day 19**	**Day 20**	**Day 21**	**Day 22**	**Day 23**	**Day 24**	**Day 25**	**Day 26**	**Day 27**
G1-Non swimming control	8.38 ±0.03	8.93 ±0.38	8.19 ±2.55#	5.99 ±0.31	4.99 ±0.13	6.37 ±2.07	4.73 ±0.74	4.84 ±0.98	4.1 ±0.81	6.52 ±1.57	5.57 ±0.28	4.14 ±0.79	5.31 ±0.36
G2-Swimming control	8.00 ±0.90	9.38 ±3.7	5.61 ±0.14*	6.12 ±3.19	5.34 ±2.5	5.19 ±2.46	6.13 ±1.5	4.86 ±1.22	4.73 ±2.38	7.53 ±1.62	5.67 ±2.93	5.16 ±2.18	6.28 ±2.87
G3-Amrete 30 ppm + Swimming	8.78 ±1.06	9.09 ±0.01	5.75 ±0.61*	7.59 ±0.16	6.65 ±0.57	5.39 ±0.82	5.49 ±0.43	4.85 ±0.07	4.73 ±0.07	7.99 ±1.35	4.39 ±1.69	4.07 ±2.21	4.6 ±2.49
G4-Amrete 125 ppm + Swimming	8.42 ±0.13	9.85 ±1.16	4.79 ±2.37*	7.97 ±0.77	7.32 ±0.06*#	8.22 ±1.89#	4.85 ±2.94	5.44 ±0.15	5.91 ±0.68	8.64 ±0.98*	5.45 ±0.36	4.87 ±0.63	5.63 ±1.35
G5-Revital-H + Swimming	7.61 ±0.39	8.72 ±0.04	7.53 ±0.31	5.21 ±0.46	3.27 ±1.04#	4.75 ±0.79	3.82 ±2.14	3.16 ±0.26*#	4.37 ±1.15	6.02 ±0.92	5.38 ±1.18	4.34 ±0.7	5.74 ±0.86

n=6; Values are in Mean±SEM ; p<0.05, * – significance with respect to G1, # – significance with respect to G2.

TABLE 6 – SUMMARY OF WEEKLY AVERAGE FEED CONSUMPTION OF MALE MICE

Groups & Doses	week 1	week 2	week 3	week 4
G1-Non swimming control	4.54 ±0.18#	1.39 ±0.86	4.13 ±0.19	5.84 ±0.07
G2-Swimming control	5.11 ±0.03*	1.21 ±0.04	3.89 ±1.1	5.67 ±0.26
G3-Amrete 30 ppm + Swimming	4.58 ±0.59#	1.26 ±0.31	4.21 ±0.97	6.19 ±1.44
G4-Amrete 125 ppm + Swimming	5.5 ±0.37*	1.17 ±0.42	4.54 ±0.6	6.85 ±1.72
G5-Revital-H + Swimming	4.32 ±0.05#	0.69 ±0.15*	4.35 ±0.29	6.49 ±0.09

n=6; Values are in Mean±SEM ; $p<0.05$, * – significance with respect to G1, # – significance with respect to G2.

TABLE 7 – SUMMARY OF WEEKLY AVERAGE FEED CONSUMPTION OF FEMALE MICE

Groups & Doses	week 1	week 2	week 3	week 4
G1-Non swimming control	3.64 ±1.79	0.59 ±0.27	4.37 ±0.67	7.1 ±0.83
G2-Swimming control	3.67 ±1.14	0.96 ±0.45	4.5 ±0.34	6.7 ±0.41
G3-Amrete 30 ppm + Swimming	4.41 ±1.9	0.78 ±0.15	4.5 ±0.44	6.73 ±1.06
G4-Amrete 125 ppm + Swimming	4.08 ±0.72	1.83 ±0.49*#	4.51 ±0.02	7.19 ±0.85
G5-Revital-H + Swimming	2.96 ±0.32	1.76 ±0.19*#	4.88 ±0.16	7.81 ±0.27

n=6; Values are in Mean±SEM ; $p<0.05$, * – significance with respect to G1, # – significance with respect to G2.

TABLE 8 – SUMMARY OF SWIM ENDURANCE TIME OF MALE MICE

Groups & Doses	Week 0 (sec)	Week 1 (sec)	Week 2 (sec)	Week 3 (sec)	Week 4 (sec)
G2-Swimming control	521.84 ±196.41	295.5 ±178.6	599.00 ±311.84	656.5 ±101.66	523.67 ±64.46
G3-Amrete 30 ppm + Swimming	548.67 ±214.91	336.17 ±251.08	533.34 ±143.39	793.5 ±171.65	636.34 ±57.00
G4-Amrete 125 ppm + Swimming	544.00 ±206.65	419.34 ±149.56	896.17 ±208.14	951.84 ±99.11	796.5 ±108.86#
G5-Revital-H + Swimming	553.67 ±218.32	389.5 ±243.56	600.5 ±143.61	842.00 ±383.77	1097.5 ±176.44#

n=6; Values are in Mean±SD ; $p<0.05$, * – significance with respect to G1, # – significance with respect to G2.

TABLE 9 - % ANTI-FATIGUE ACTIVITY OF MALE MICE

Groups & Doses	Doses	Anti-fatigue Activity (%)				
		Week 0	Week 1	Week 2	Week 3	Week 4
G2-Swimming control	0	0.00	0.00	0.00	0.00	0.00
G3-Amrete 30 ppm + Swimming	30 ppm	5.14	13.76	-10.96	20.87	21.51
G4-Amrete 125 ppm + Swimming	125 ppm	4.25	41.91	49.61	44.99	52.10
G5-Revital-H + Swimming	200 (mg/kg)	6.10	31.81	0.25	28.26	109.58

Calculated based on Mean swim endurance time

TABLE 10 – SUMMARY OF SWIM ENDURANCE TIME OF FEMALE MICE

Groups & Doses	Week 0 (sec)	Week 1 (sec)	Week 2 (sec)	Week 3 (sec)	Week 4 (sec)
G2-Swimming control	536.84 ±179.24	580.00 ±101.2	734.17 ±165.71	407.17 ±92.9	547.34 ±149.95
G3-Amrete 30 ppm + Swimming	546.00 ±190.29	479.67 ±149.03	657.17 ±264.99	407.67 ±85.99	714.17 ±66.72
G4-Amrete 125 ppm + Swimming	549.34 ±203.52	393.5 ±140.74	875.00 ±262.64	470.5 ±120.21	916.17 ±91.94#
G5-Revital-H + Swimming	533.17 ±181.67	463.5 ±145.37	844.34 ±249.4	601.5 ±81.18	1081.84 ±138.39#

n=6; Values are in Mean±SD; p<0.05, * – significance with respect to G1, # – significance with respect to G2.

TABLE 11 - % ANTI-FATIGUE ACTIVITY OF FEMALE MICE

Groups & Doses	Anti-fatigue Activity (%)				
	Week 0	Week 1	Week 2	Week 3	Week 4
G2, Swimming control	0.00	0.00	0.00	0.00	0.00
G3, Amrete 30ppm + Swimming	1.71	-17.30	-10.49	0.12	30.48
G4, Amrete 125 ppm + Swimming	2.33	-32.16	19.18	15.55	67.39
G5, RI-Revital-H + Swimming	-0.68	-20.09	15.01	47.73	97.66

Calculated based on Mean swim endurance time

TABLE 12 - SUMMARY OF CLINICAL CHEMISTRY PARAMETERS OF MALE MICE

Groups & Doses	pH	Pco2 mmHg	Po2 mmHg	Chco3 mmol/L	BE(ecf) mmol/L	Na+ mmol/L	K+ mmol/L	Ca++ mmol/L	Cl+ mmol/L
G1-Non Swimming control	7.02 ±0.22	62.45 ±22.07	30.79 ±22.64#	15.09 ±3.7#	-15.95 ±5.68	150.67 ±2.26	5.20 ±1.43	1.05 ±0.15	121.5 ±3.62
G2-Swimming control	7.15 ±0.15	28.07 ±9.33*#	61.44 ±18.01*	9.79 ±3.29*	-19.14 ±5.22	144.67 ±10.2	4.64 ±1.24	0.86 ±0.13	125.84 ±4.88
G3-Amrete 30 ppm + Swimming	7.07 ±0.09	34.14 ±6.44*	57.97 ±15.91*	9.67 ±1.37*	-20.62 ±2.48	148.17 ±1.48	5.44 ±0.84	0.92 ±0.17	124.5 ±4.55
G4-Amrete 125 ppm + Swimming	7.10 ±0.13	42.77 ±7.18	34.77 ±9.61#	13.09 ±2.47	-16.72 ±4.43	148.00 ±2.29	4.94 ±1.06	0.85 ±0.17	122.5 ±3.51
G5-Revital-H + Swimming	7.08 ±0.09	40.30 ±15.23*	35.15 ±18.58	11.52 ±2.88	-18.50 ±2.91	128.95 ±49.63	5.39 ±1.31	0.92 ±0.10	124.17 ±2.57

n=6; Values are in Mean±SD; p<0.05, * – significance with respect to G1, # – significance with respect to G2.

CONTD., TABLE 12 - SUMMARY OF CLINICAL CHEMISTRY PARAMETERS OF MALE MICE

Groups & Doses	Ctco2 mmol/L	Hgb g/dl	Glucose mg/dl	Lactate mmol/L	Creatinine mg/dl	Urea mg/dl	CK U/L	LDH U/L
G1-Non Swimming control	17.02 ±3.88#	10.30 ±1.12	147.34 ±41.19	11.46 ±4.02	0.45 ±0.26	45.42 ±8.01#	837.06 ±943.24	1611.20 ±966.91
G2-Swimming control	10.62 ±3.38*	8.42 ±1.30	99.34 ±29.35	8.02 ±1.37	0.35 ±0.12	62.56 ±15.8*	672.52 ±637.75	4500.00 ±817.31
G3-Amrete 30 ppm + Swimming	10.70 ±1.43*	8.77 ±2.33	140.5 ±67.88	10.99 ±1.68	0.30 ±0.00	57.39 ±10.73	720.30 ±438.65	4321.80 ±1861.56
G4-Amrete 125 ppm + Swimming	14.40 ±2.38	8.90 ±2.00	74.00 ±36.83*	8.05 ±1.64	0.30 ±0.00	58.77 ±7.21	553.56 ±413.32	3078.60 ±1663.52
G5-Revital-H + Swimming	11.92 ±3.34*	9.54 ±2.61	122.84 ±44.26	10.22 ±2.66	0.37 ±0.16	62.00 ±7.34*	498.90 ±178.62	2889.80 ±1190.18

n=6; Values are in Mean±SD; p<0.05, * – significance with respect to G1, # – significance with respect to G2.

TABLE 13 - SUMMARY OF CLINICAL CHEMISTRY PARAMETERS OF FEMALE MICE

Groups & Doses	pH	Pco2 mmHg	Po2 mmHg	Chco3 mmol/L	BE(ecf) mmol/L	Na+ mmol/L	K+ mmol/L	Ca++ mmol/L	Cl+ mmol/L
G1-Non Swimming control	7.11 ±0.11	36.99 ±21.21	56.25 ±18.79	10.89 ±4.61	-18.62 ±4.31	151.67 ±1.37	4.67 ±0.46	0.93 ±0.19	126.00 ±6.42
G2-Swimming control	7.14 ±0.13	29.1 ±17.28	50.67 ±18.82	9.04 ±3.44	-20.05 ±2.79	149.5 ±1.98	4.37 ±0.63	0.92 ±0.12	125.84 ±3.00
G3-Amrete 30 ppm + Swimming	7.12 ±0.09	28.6 ±10.43	45.84 ±21.77	8.80 ±1.44	-20.59 ±0.68	148.17 ±3.61	4.74 ±0.63	0.88 ±0.07	123.67 ±3.39
G4-Amrete 125 ppm + Swimming	7.22 ±0.08	21.82 ±15.09	58.75 ±18.79	8.52 ±4.77	-19.32 ±4.65	149.84 ±1.61	3.97 ±0.96	0.82 ±0.28	127.84 ±5.78
G5-Revital-H + Swimming	7.12 ±0.09	19.55 ±6.10	55.80 ±16.55	6.50 ±1.63	-22.87 ±2.36	149.84 ±2.64	5.09 ±0.70	0.79 ±0.12	129.00 ±2.20

n=6; Values are in Mean±SD; p<0.05, * – significance with respect to G1, # – significance with respect to G2.

CONTD., TABLE 13 - SUMMARY OF CLINICAL CHEMISTRY PARAMETERS OF FEMALE MICE

Groups & Doses	Cl+ mmol/L	Ctco2 mmol/L	Hgb g/dl	Glucose mg/dl	Lactate mmol/L	Creatinine mg/dl	Urea mg/dl	CK U/L	LDH U/L
G1-Non Swimming control	126.00 ±6.42	12.10 ±5.26	7.17 ±3.33	122.17 ±38.89	10.89 ±2.64	0.32 ±0.03	52.30 ±10.14	450.56 ±211.70	1691.00 ±354.21
G2-Swimming control	125.84 ±3.00	9.95 ±3.97	7.95 ±1.02	132.50 ±29.78	9.46 ±2.18	0.32 ±0.03	61.98 ±15.92	504.08 ±255.97	1028.20 ±246.16
G3-Amrete 30 ppm + Swimming	123.67 ±3.39	9.69 ±1.76	7.80 ±0.48	107.84 ±35.47	10.03 ±1.74	0.32 ±0.03	68.57 ±10.52	479.40 ±134.95	1656.40 ±915.26
G4-Amrete 125 ppm + Swimming	127.84 ±5.78	9.17 ±5.21	6.80 ±2.94	109.00 ±47.89	7.81 ±2.76	0.31 ±0.01	61.35 ±12.20	432.00 ±162.73	1026.80 ±131.63
G5-Revital-H + Swimming	129.00 ±2.20	7.10 ±1.76	6.47 ±1.35	93.00 ±36.09	9.34 ±2.41	0.32 ±0.03	66.15 ±6.84	519.16 ±152.47	2322.34 ±1639.10

n=6; Values are in Mean±SD; p<0.05, * – significance with respect to G1, # – significance with respect to G2.

TABLE 14 - SUMMARY OF LIVER & MUSCLE GLYCOGEN AND TISSUE ATP LEVELS

Groups	Glycogen and ATP					
	Male			Female		
	Liver Glycogen (µg/mg)	Muscle Glycogen (µg/mg)	Muscle ATP (nmol/mg)	Liver Glycogen (µg/mg)	Muscle Glycogen (µg/mg)	Muscle ATP (nmol/mg)
G1-Non Swimming control	13.31 ±0.35$^{\#}$	3.49 ±0.19$^{\#}$	0.80 ±0.11	12.73 ±0.27$^{\#}$	3.35 ±0.21	0.97 ±0.10
G2-Swimming control	11.16 ±0.88*	2.77 ±0.11*	0.63 ±0.05	10.74 ±0.56*	2.92 ±0.18	0.75 ±0.16
G3-Amrete 30 ppm + Swimming	14.96 ±0.66$^{*\#}$	5.21 ±0.44$^{*\#}$	1.17 ±0.14$^{*\#}$	13.81 ±0.77$^{*\#}$	4.34 ±0.44$^{*\#}$	1.19 ±0.15$^{\#}$
G4-Amrete 125 ppm + Swimming	17.14 ±0.61$^{*\#}$	7.60 ±0.25$^{*\#}$	1.58 ±0.17$^{*\#}$	16.16 ±0.28$^{*\#}$	7.34 ±0.25$^{*\#}$	1.72 ±0.15$^{*\#}$
G5-Revital-H + Swimming	19.45 ±0.55$^{*\#}$	10.30 ±0.24$^{*\#}$	2.14 ±0.02$^{*\#}$	18.46 ±0.24$^{*\#}$	9.45 ±0.32$^{*\#}$	2.09 ±0.20$^{*\#}$

n=6; Values are in Mean±SD; $p<0.05$, * – significance with respect to G1, $^{\#}$ – significance with respect to G2.

9. FIGURES

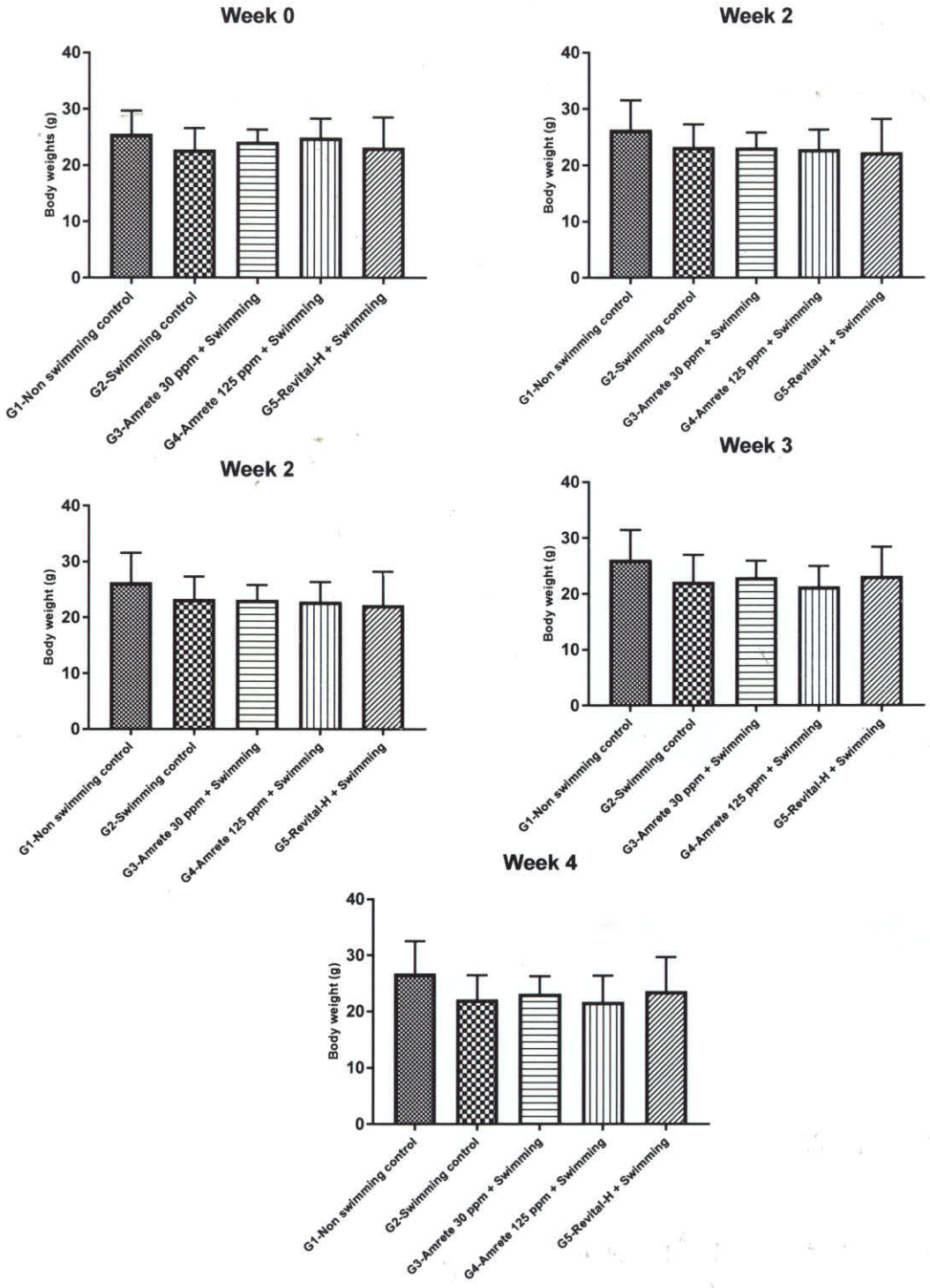

Fig 1 : Summary of Body weights in males.

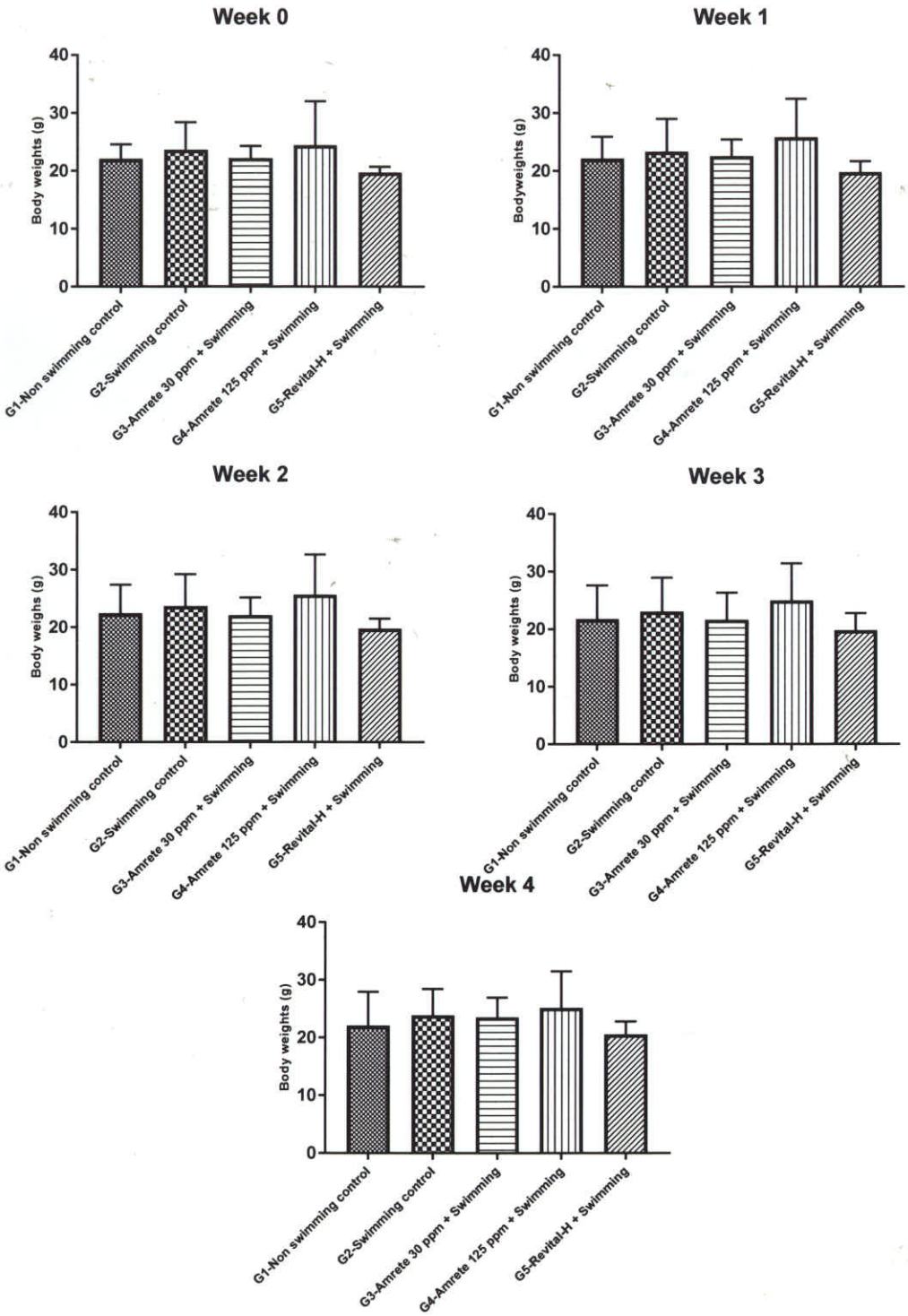

Fig 2: Summary of Body weights in females

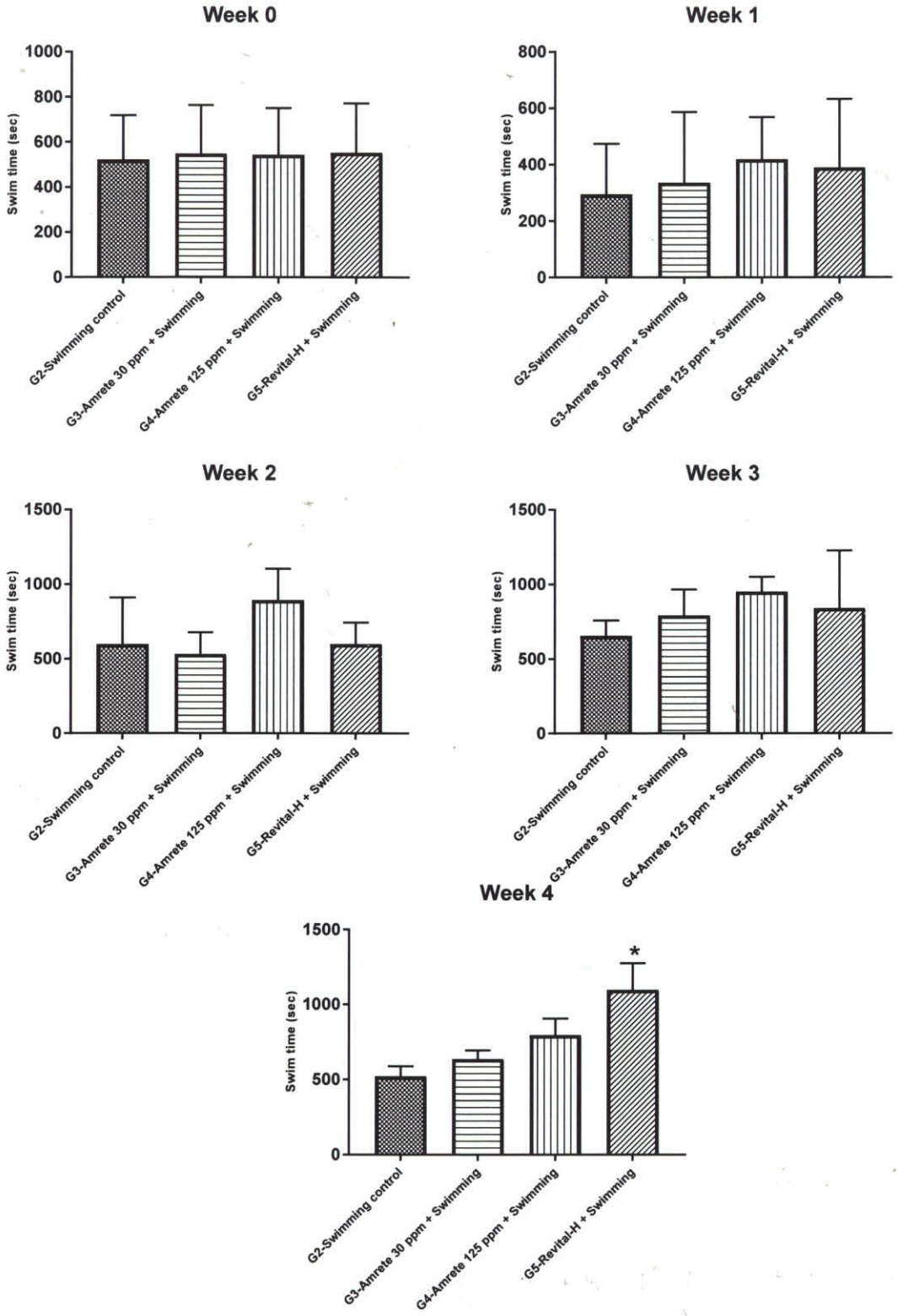

Fig 3: Summary of Swimming time in males

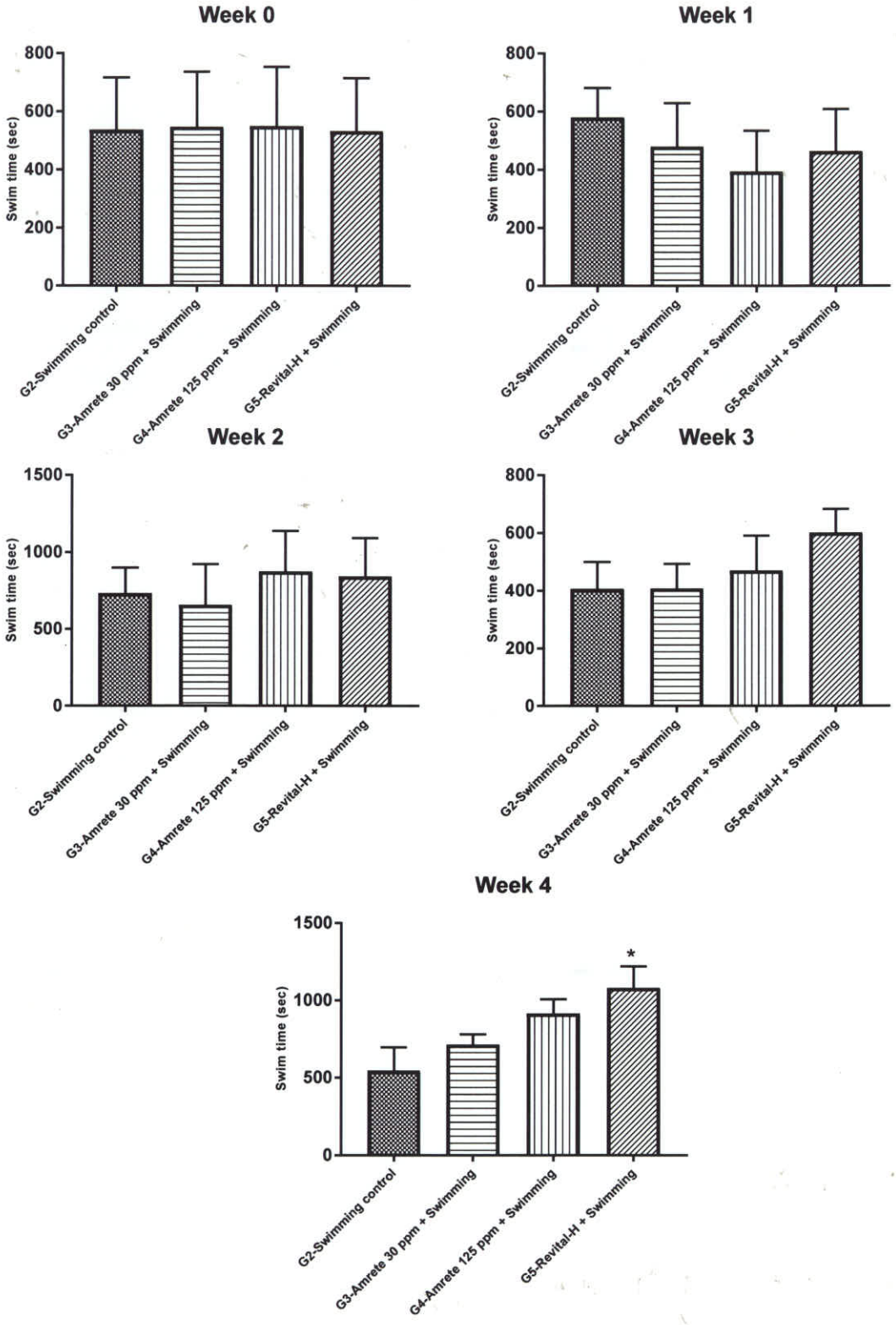

Fig 4: Summary of Swimming time in females

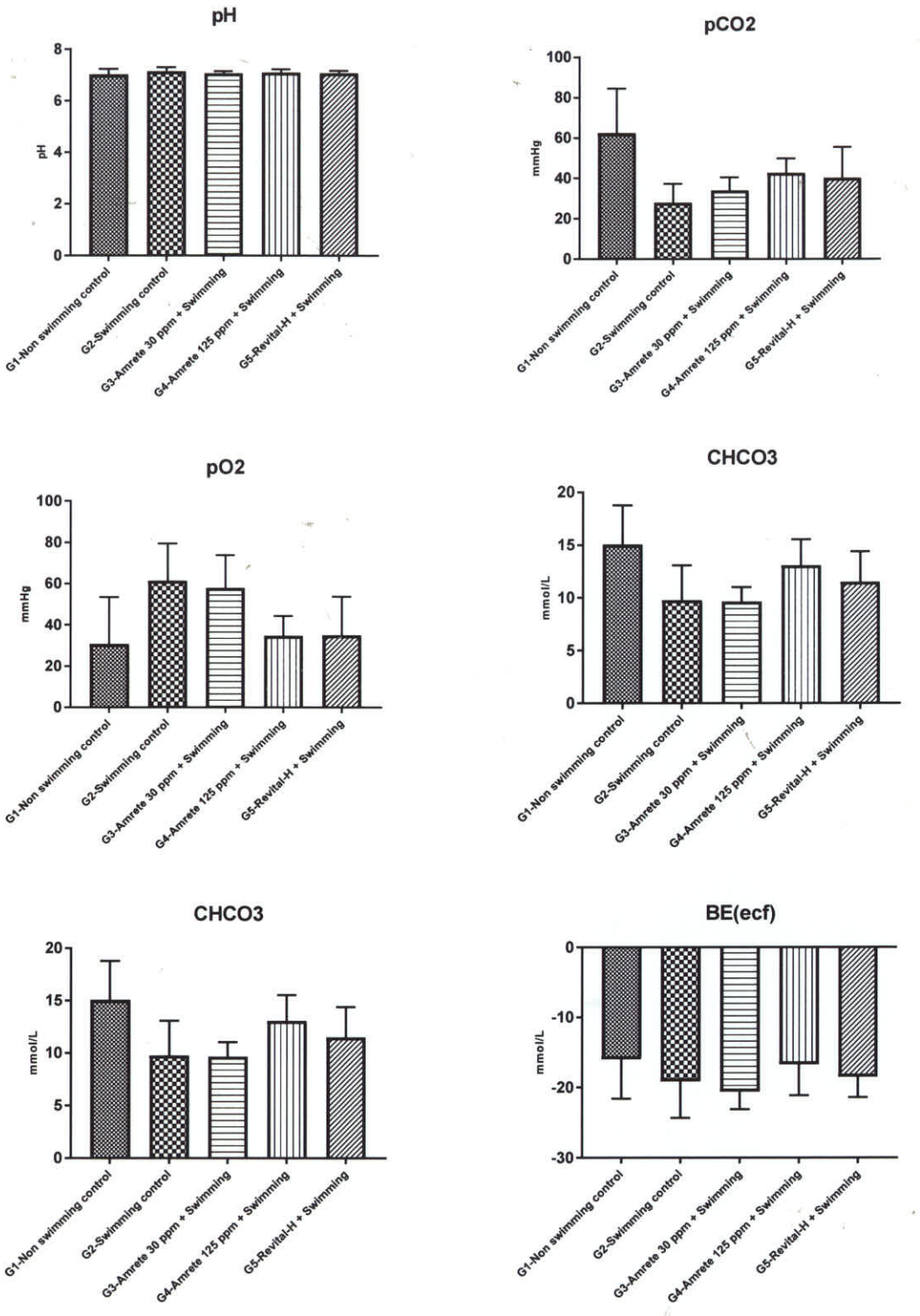

Fig 5: Summary of Clinical pathology in males

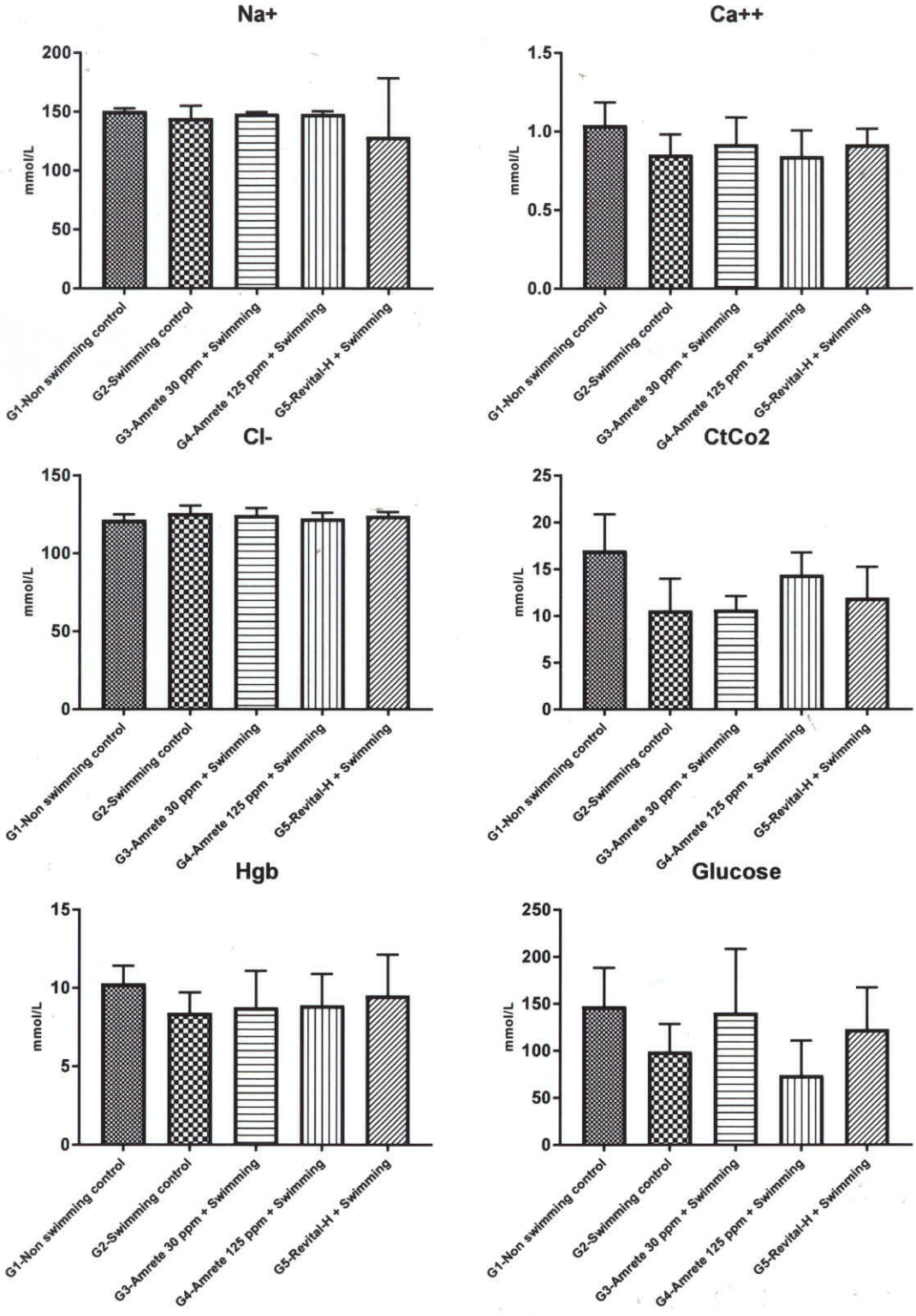

Fig 5: Contd., Summary of Clinical pathology in males

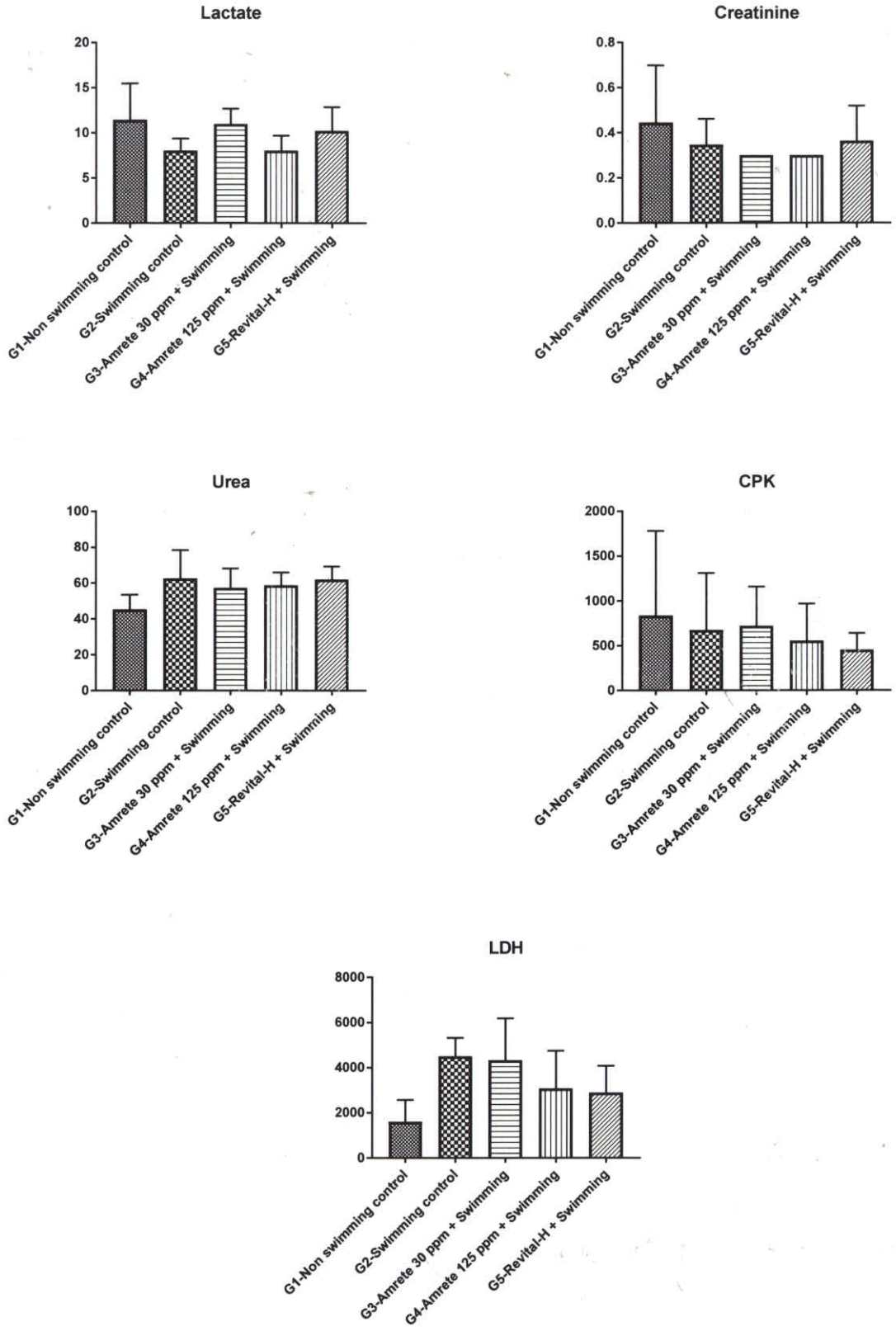

Fig 5: Contd., Summary of Clinical pathology in males

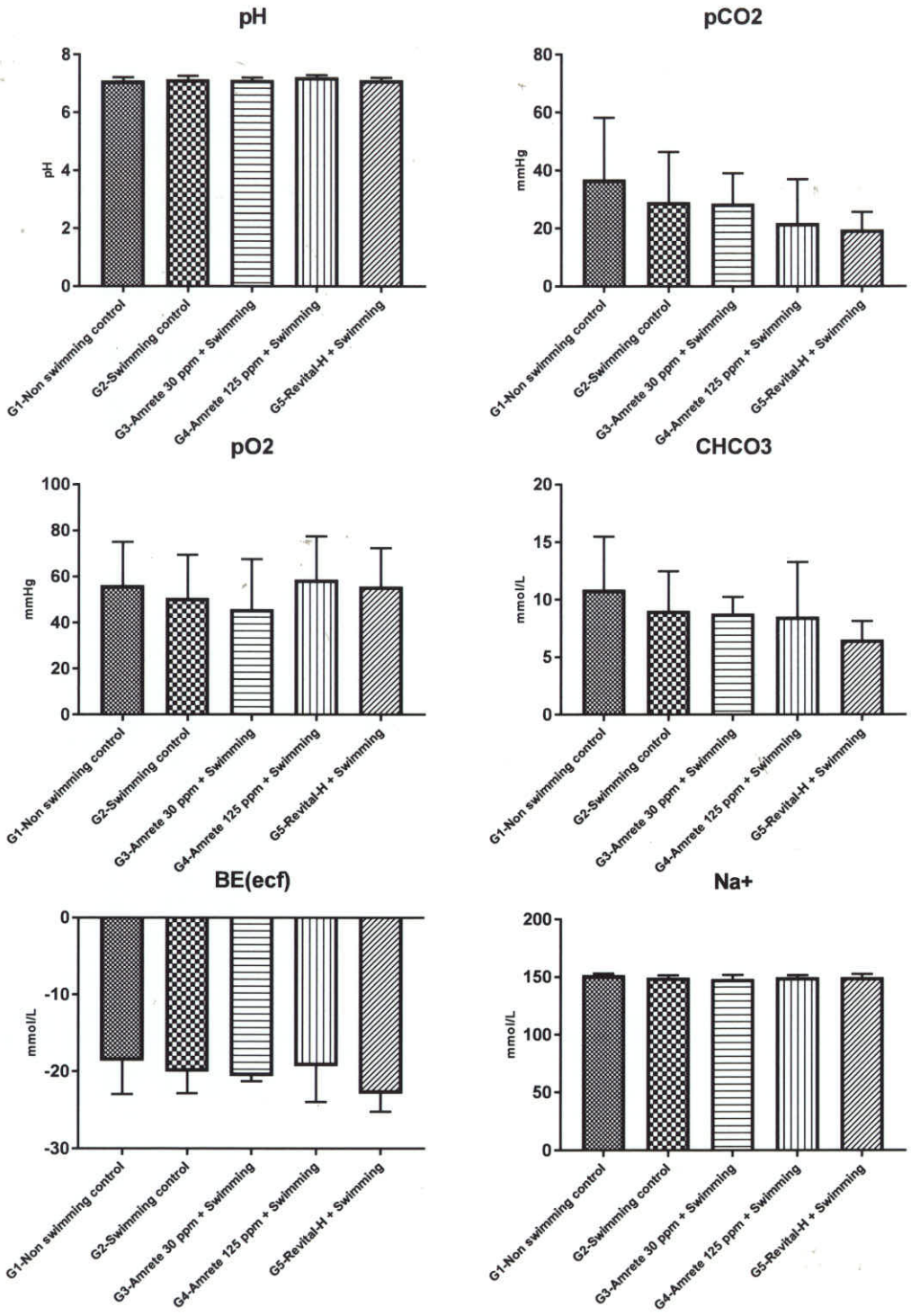

Fig 6: Summary of Clinical pathology in females

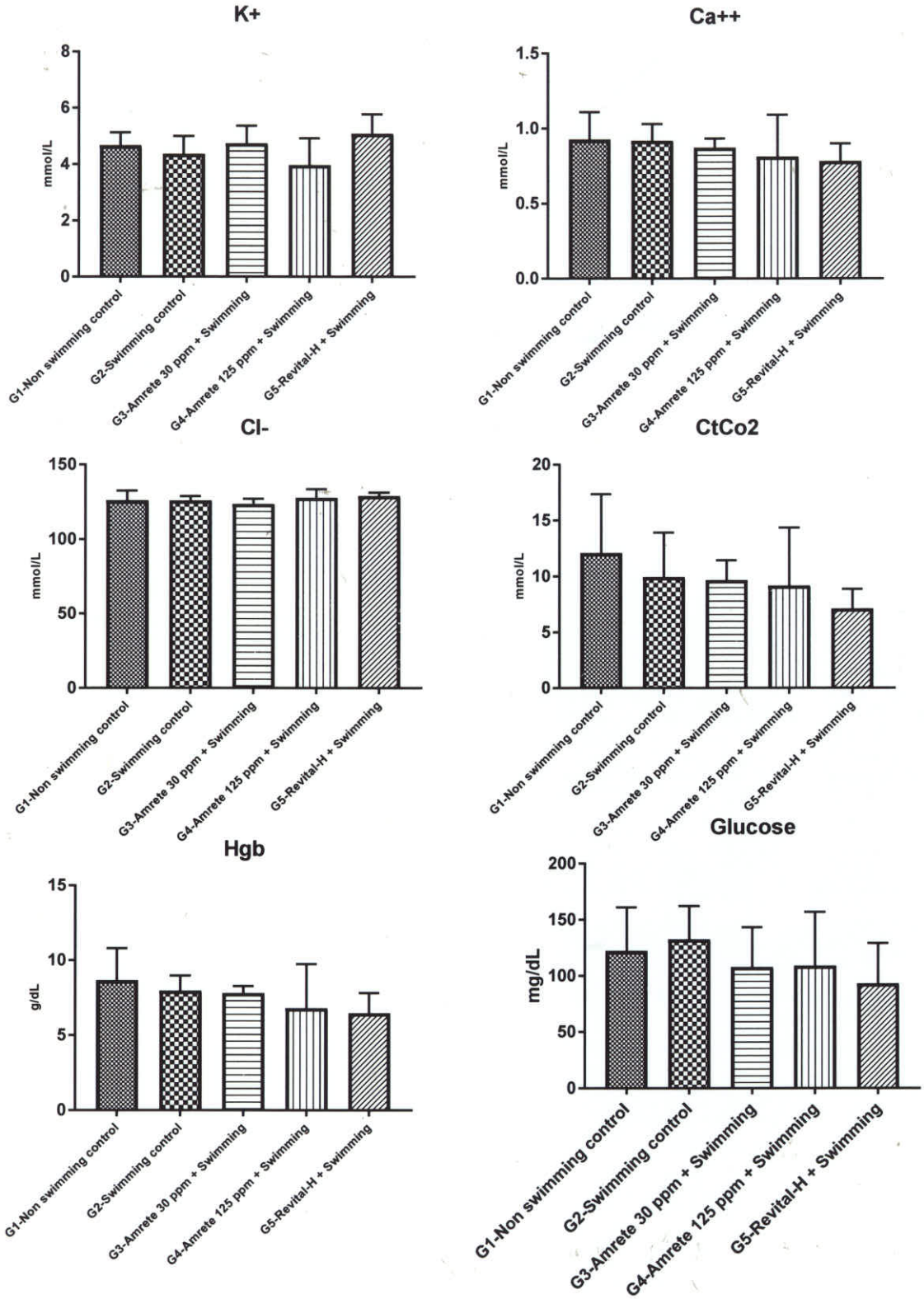

Fig 6: Contd., Summary of Clinical pathology in females

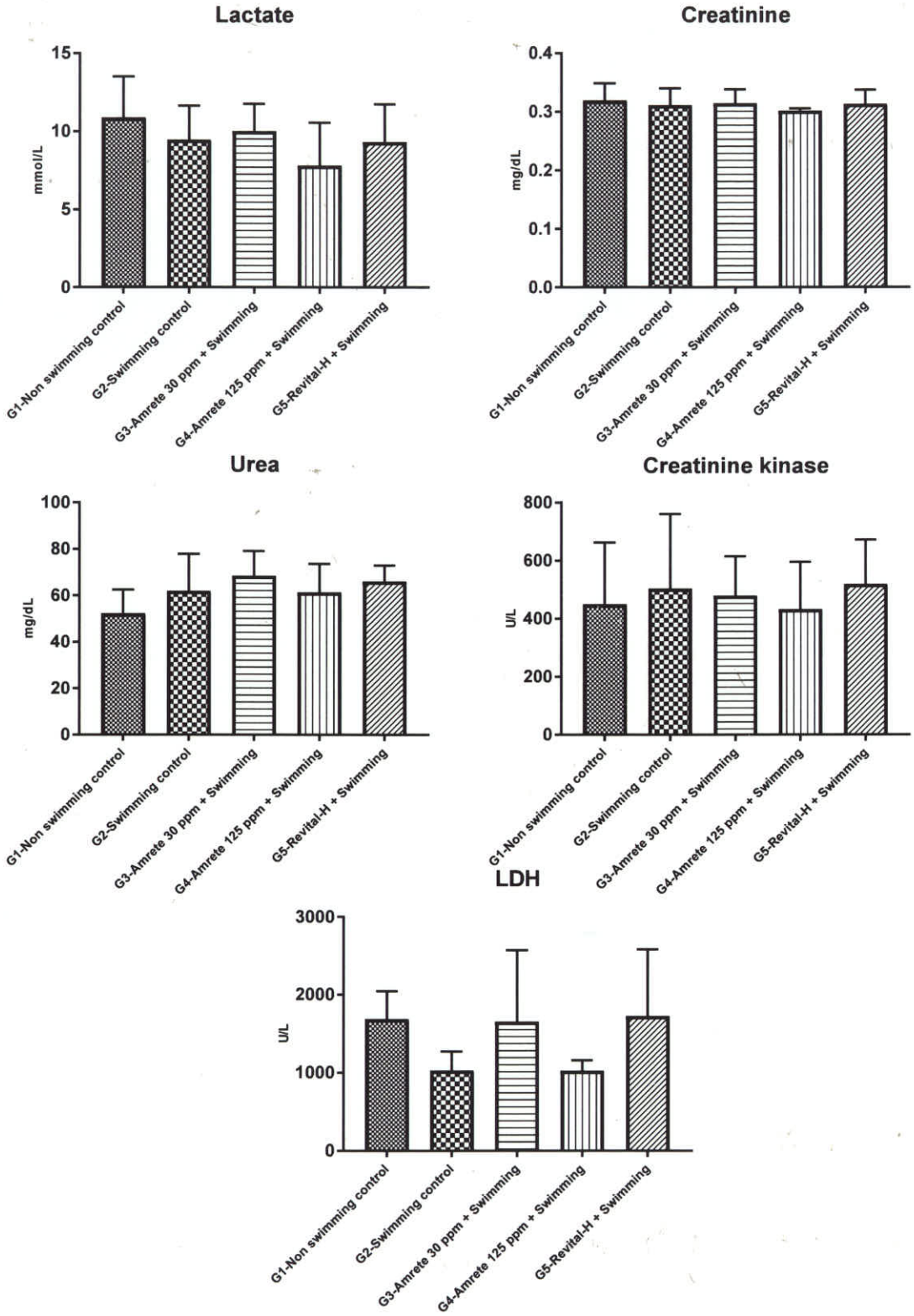

Fig 6: Contd., Summary of Clinical pathology in females

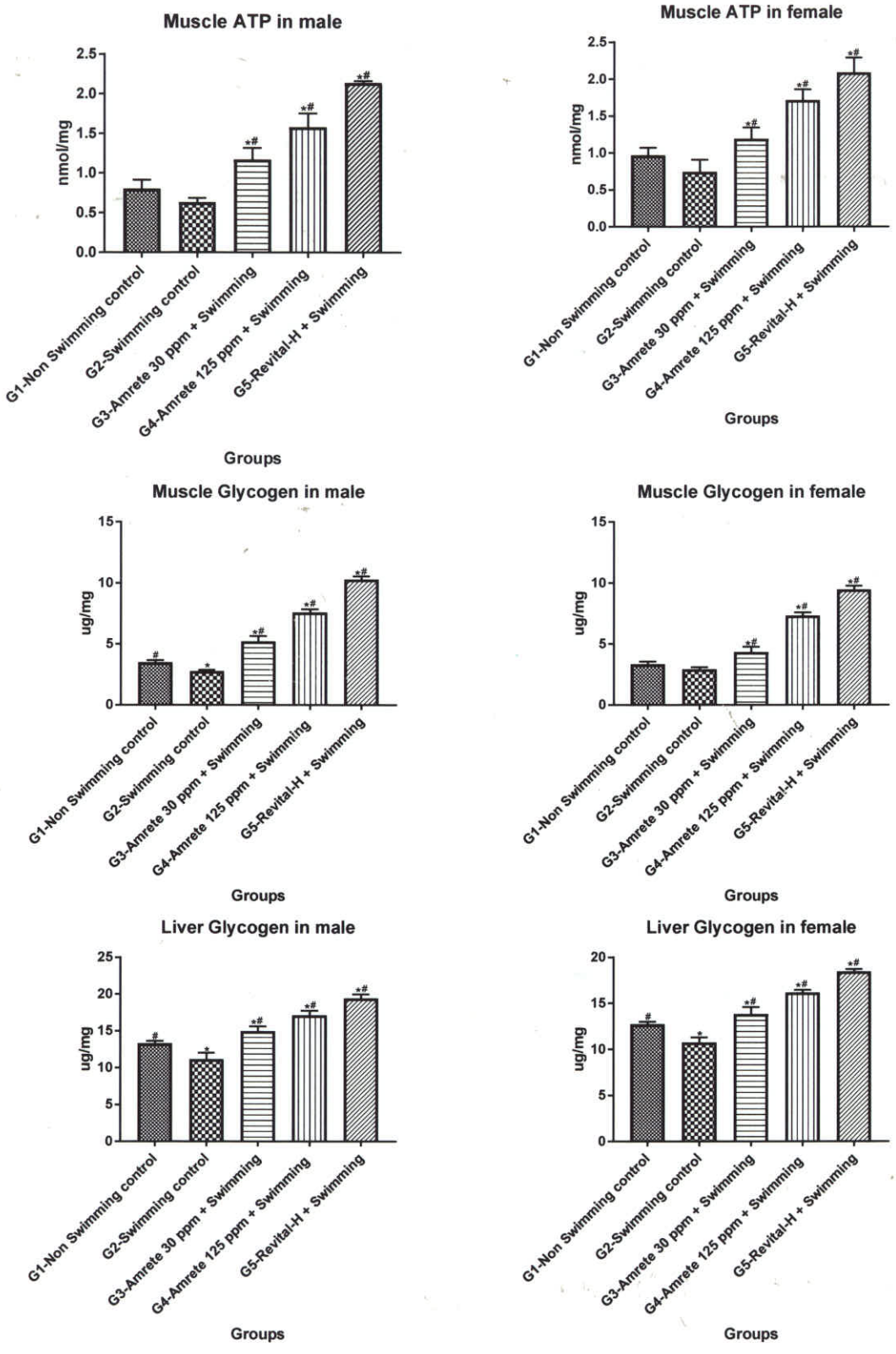

Fig 7: Summary of liver & muscle glycogen and tissue ATP levels

10. APPENDICES

APPENDIX 1 - INDIVIDUAL ANIMAL CLINICAL SIGNS, DETAILED CLINICAL EXAMINATION AND MORTALITY OF MALES

Groups	Animal No.	Days																											
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
G1-Non Swimming control	01	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	02	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	03	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	04	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	05	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	06	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
G2-Swimming control	07	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	08	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	09	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	11	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
G3-Amrete 30 ppm + Swimming	13	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	17	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	18	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
G4-Amrete 125 ppm + Swimming	19	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	21	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	22	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	23	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	24	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
G5-Revital-H + Swimming	25	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	26	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	27	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	28	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	29	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	30	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

1= No Abnormalities Detected,

APPENDIX 2 - INDIVIDUAL ANIMAL CLINICAL SIGNS, DETAILED CLINICAL EXAMINATION AND MORTALITY OF FEMALES

Groups	Animal No.	Days																										
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
G1-Non Swimming control	31	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	32	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	33	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	34	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	35	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	36	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
G2- Swimming control	37	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	38	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	39	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	40	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	41	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	42	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
G3-Amrete 30 ppm + Swimming	43	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	44	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	45	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	46	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	47	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	48	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
G4-Amrete 125 ppm + Swimming	49	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	50	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	51	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	52	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	53	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	54	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
G5-Revital-H + Swimming	55	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	56	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	57	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	58	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	59	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	60	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

1= No Abnormalities Detected,

APPENDIX 3 - INDIVIDUAL ANIMAL BODY WEIGHT OF MALE MICE

Groups	Animal No.	BODY WEIGHT (g)				
		Week 0	Week 1	Week 2	Week 3	Week 4
G1-Non Swimming control	01	19.87	18.55	20.08	20.12	20.78
	02	24.61	26.14	26.24	25.51	26.51
	03	29.28	31.58	31.08	31.47	31.93
	04	22.97	22.43	20.93	20.39	19.64
	05	31.14	33.95	33.13	32.51	34.11
	06	25.40	27.26	26.48	26.85	27.45
G2-Swimming control	07	25.21	26.36	26.01	24.05	23.17
	08	18.84	19.84	19.27	15.18	17.10
	09	23.77	23.39	24.40	24.05	23.41
	10	26.46	27.00	27.12	26.53	26.57
	11	25.14	25.15	25.50	26.09	25.94
	12	17.21	14.79	17.31	16.92	16.67
G3-Amrete 30 ppm + Swimming	13	24.96	21.45	20.30	19.45	20.44
	14	24.08	24.12	24.74	26.79	27.20
	15	21.95	24.29	22.07	19.23	18.66
	16	21.89	23.02	23.24	23.66	23.75
	17	24.25	24.57	21.07	23.47	23.95
	18	27.73	28.33	27.50	25.00	25.02
G4-Amrete 125 ppm + Swimming	19	30.43	30.41	29.27	28.44	28.62
	20	26.05	23.62	21.62	20.66	18.68
	21	21.02	19.48	19.37	18.65	18.18
	22	21.81	22.91	23.77	21.45	26.67
	23	25.08	26.29	22.65	19.62	19.27
	24	25.06	20.74	20.52	19.14	18.89
G5-Revital-H + Swimming	25	17.87	16.36	18.45	21.17	18.29
	26	21.77	20.13	21.32	25.41	26.14
	27	33.56	32.65	34.05	32.70	34.62
	28	21.26	19.42	18.36	18.36	19.12
	29	22.72	21.12	20.09	21.12	22.17
	30	21.84	20.04	21.31	20.36	21.36

APPENDIX 4 - INDIVIDUAL ANIMAL BODY WEIGHT OF FEMALE MICE

Groups	Animal No.	BODY WEIGHT (g)				
		Week 0	Week 1	Week 2	Week 3	Week 4
G1-Non Swimming control	31	20.63	21.49	22.12	22.79	23.40
	32	23.69	24.19	26.76	28.19	26.95
	33	19.48	16.02	16.80	15.62	15.31
	34	24.05	26.15	27.64	28.18	29.86
	35	19.80	24.95	25.08	21.06	20.99
	36	25.14	20.16	16.32	14.86	15.92
G2-Swimming control	37	24.57	26.41	27.74	24.60	25.68
	38	27.38	27.67	28.83	28.50	28.41
	39	28.45	27.23	27.83	29.28	27.94
	40	15.16	12.74	16.00	17.32	19.44
	41	23.66	24.31	23.86	23.87	24.52
	42	22.70	21.74	17.89	14.67	17.28
G3-Amrete 30 ppm + Swimming	43	23.64	24.75	24.86	23.83	22.84
	44	20.99	21.60	19.96	17.79	19.76
	45	19.65	20.84	20.91	20.93	19.70
	46	23.11	24.41	24.24	24.29	24.39
	47	25.07	25.52	24.99	27.88	27.97
	48	20.92	18.19	17.69	15.03	26.53
G4-Amrete 125 ppm + Swimming	49	33.41	33.51	33.87	30.90	30.67
	50	22.34	22.75	20.17	16.80	17.27
	51	24.43	24.37	24.16	23.02	23.82
	52	17.18	20.98	21.20	22.67	22.76
	53	19.25	18.70	18.62	19.49	20.30
	54	19.96	24.55	26.14	27.26	26.35
G5-Revital-H + Swimming	55	19.25	20.83	20.66	21.43	21.63
	56	19.96	19.52	19.50	20.84	21.26
	57	20.11	19.34	18.62	16.42	19.59
	58	19.33	18.76	21.54	22.97	22.98
	59	21.25	22.81	21.17	21.44	21.46
	60	18.41	17.29	17.06	15.63	16.57

APPENDIX 5 - INDIVIDUAL ANIMAL AVERAGE WATER CONSUMPTION

Groups	Average water Consumption (mL/Mice/day)										
	Days										
	Sex	An. No.	01	02	03	04	05	06	07	08	09
G1-Non Swimming control	M	1,2,3	4.93	3.48	4.40	4.12	4.65	3.65	4.32	3.89	4.44
	M	4,5,6	4.36	3.94	3.66	3.75	6.27	5.71	5.93	6.30	5.03
	F	31,32,33	2.71	1.88	3.10	3.22	2.91	3.92	4.00	4.09	2.84
	F	34,35,36	3.54	2.86	4.06	4.18	3.42	2.95	3.22	2.92	2.58
G2-Swimming control	M	7,8,9	3.99	2.75	3.82	4.83	5.09	5.23	5.25	5.52	4.87
	M	10,11,12	3.91	3.02	2.58	3.75	3.90	3.03	2.38	2.13	1.20
	F	37,38,39	4.16	3.28	3.43	3.56	4.61	4.02	3.87	3.19	4.05
	F	40,41,42	4.22	3.35	3.91	3.14	3.40	3.56	3.55	3.18	3.79
G3-Amrete 30 ppm + Swimming	M	13,14,15	4.34	3.10	3.40	5.09	6.54	5.01	4.04	6.11	4.53
	M	16,17,18	4.03	3.34	3.88	4.02	4.74	3.72	4.23	4.32	3.43
	F	43,44,45	2.72	1.51	2.81	3.92	3.12	3.51	3.25	2.80	2.06
	F	46,47,48	3.73	1.29	2.36	4.17	3.48	3.98	4.32	4.19	3.56
G4-Amrete 125 ppm + Swimming	M	19,20,21	6.17	3.99	4.23	4.29	4.96	3.76	3.90	5.08	4.29
	M	22,23,24	5.01	3.77	4.68	4.98	4.47	4.00	4.66	4.65	3.95
	F	49,50,51	3.80	6.41	7.88	7.15	6.94	7.93	7.54	6.50	6.37
	F	52,53,54	3.98	1.46	3.14	3.78	4.20	4.74	5.17	4.52	3.20
G5-Revital-H + Swimming	M	25,26,27	6.55	3.21	4.89	5.56	6.37	3.95	4.86	5.19	3.72
	M	28,29,30	4.04	2.60	3.03	3.49	3.99	2.16	2.23	3.34	2.84
	F	55,56,57	1.60	1.94	3.43	2.05	2.53	2.88	3.61	2.88	2.89
	F	58,59,60	1.84	2.43	3.15	3.51	2.15	2.93	3.33	2.49	2.00

APPENDIX 5 CONTD., - INDIVIDUAL ANIMAL AVERAGE WATER CONSUMPTION

Groups	Average water Consumption (mL/Mice/day)										
	Days										
	Sex	An. No.	10	11	12	13	14	15	16	17	18
G1-Non Swimming control	M	1,2,3	4.60	3.64	4.62	7.24	7.86	5.49	8.45	6.28	7.70
	M	4,5,6	5.94	8.26	3.73	8.14	8.31	6.11	8.58	7.81	7.94
	F	31,32,33	4.42	4.30	8.44	7.66	9.27	8.36	8.66	6.39	5.77
	F	34,35,36	4.48	5.20	6.43	9.35	9.25	8.40	9.20	9.98	6.20
G2-Swimming control	M	7,8,9	4.69	5.60	6.95	7.54	9.15	7.89	10.75	7.76	8.87
	M	10,11,12	4.54	4.98	4.76	4.77	10.12	4.54	8.62	6.12	7.25
	F	37,38,39	3.25	6.77	7.77	11.00	9.24	8.63	11.99	5.51	8.37
	F	40,41,42	4.65	5.00	9.39	10.40	8.95	7.37	6.76	5.70	3.86
G3-Amrete 30 ppm + Swimming	M	13,14,15	5.07	5.11	2.65	4.56	7.33	7.23	7.84	6.13	9.27
	M	16,17,18	4.00	5.75	5.61	5.75	7.57	7.18	7.76	7.26	8.36
	F	43,44,45	3.96	3.99	8.00	7.13	7.60	8.03	9.09	5.32	7.48
	F	46,47,48	4.92	4.47	7.10	9.96	8.65	9.52	9.08	6.18	7.69
G4-Amrete 125 ppm + Swimming	M	19,20,21	4.02	5.13	4.87	6.32	6.90	6.90	8.76	8.57	6.82
	M	22,23,24	3.98	5.50	5.53	6.05	6.69	6.71	7.76	7.54	7.03
	F	49,50,51	7.32	3.33	6.79	6.60	9.87	8.51	10.66	3.11	8.51
	F	52,53,54	6.36	6.19	7.14	8.27	7.16	8.33	9.03	6.46	7.42
G5-Revital-H + Swimming	M	25,26,27	5.85	4.14	3.82	6.35	8.95	6.89	9.41	9.38	5.92
	M	28,29,30	2.99	3.07	3.17	5.44	7.54	7.04	8.57	7.94	7.94
	F	55,56,57	4.08	4.54	6.28	6.79	7.43	7.33	8.74	7.31	4.89
	F	58,59,60	3.50	3.31	6.35	7.92	7.14	7.87	8.69	7.74	5.53

APPENDIX 5 CONTD., - INDIVIDUAL ANIMAL AVERAGE WATER CONSUMPTION

Groups	Average water Consumption (mL/Mice/day)										
	Days										
	Sex	An. No.	19	20	21	22	23	24	25	26	27
G1-Non Swimming control	M	1,2,3	6.43	5.08	6.78	6.54	5.40	5.80	6.89	5.79	5.47
	M	4,5,6	6.35	5.66	6.08	5.59	4.81	5.14	7.48	6.87	5.00
	F	31,32,33	4.90	7.83	4.20	5.53	4.67	7.62	5.76	4.69	5.56
	F	34,35,36	5.08	4.91	5.24	4.15	3.52	5.41	5.37	3.58	5.06
G2-Swimming control	M	7,8,9	5.00	5.36	5.02	4.69	3.90	3.16	5.11	3.08	4.19
	M	10,11,12	2.80	2.66	5.64	2.39	3.58	3.58	5.68	4.29	3.79
	F	37,38,39	7.10	6.92	7.18	5.72	6.41	8.67	7.74	6.70	8.30
	F	40,41,42	3.57	3.45	5.07	4.00	3.04	6.38	3.60	3.62	4.25
G3-Amrete 30 ppm + Swimming	M	13,14,15	7.21	6.96	5.56	5.10	5.02	5.29	8.12	4.63	4.78
	M	16,17,18	6.15	5.28	5.35	4.94	4.73	4.92	7.28	3.29	3.13
	F	43,44,45	6.24	4.81	5.18	4.79	4.78	7.04	3.19	2.50	2.84
	F	46,47,48	7.04	5.96	5.78	4.89	4.68	8.93	5.58	5.62	6.35
G4-Amrete 125 ppm + Swimming	M	19,20,21	5.81	6.81	5.70	5.63	4.86	4.88	7.19	3.95	3.26
	M	22,23,24	5.10	4.89	15.03	6.82	4.41	4.04	6.90	3.83	3.35
	F	49,50,51	7.35	9.55	2.77	5.34	6.38	9.32	5.70	5.31	6.57
	F	52,53,54	7.28	6.88	6.93	5.54	5.43	7.95	5.19	4.42	4.67
G5-Revital-H + Swimming	M	25,26,27	5.67	5.77	5.22	4.91	3.85	4.47	6.47	6.16	4.49
	M	28,29,30	4.99	4.51	6.58	6.48	4.53	3.85	6.38	4.91	3.58
	F	55,56,57	2.53	4.19	2.30	2.97	5.17	6.66	6.21	4.83	6.34
	F	58,59,60	3.99	5.30	5.32	3.33	3.56	5.37	4.54	3.85	5.13

APPENDIX 6 - INDIVIDUAL ANIMAL AVERAGE FEED CONSUMPTION

Groups	Average Food Consumption (g/Mice/day)					
			Week			
	Sex	An. No.	1	2	3	4
G1-Non Swimming control	M	1,2,3	4.66	0.78	4.26	5.78
	M	4,5,6	4.41	1.98	3.99	5.88
	F	31,32,33	2.38	0.77	4.84	7.68
	F	34,35,36	4.90	0.41	3.90	6.51
G2- Swimming control	M	7,8,9	5.09	1.23	4.66	5.84
	M	10,11,12	5.12	1.18	3.11	5.49
	F	37,38,39	4.47	1.27	4.73	6.98
	F	40,41,42	2.86	0.64	4.26	6.40
G3- Amrete 30 ppm + Swimming	M	13,14,15	4.16	1.04	4.89	7.20
	M	16,17,18	4.99	1.47	3.53	5.17
	F	43,44,45	3.07	0.67	4.81	7.47
	F	46,47,48	5.74	0.88	4.19	5.98
G4- Amrete 125 ppm + Swimming	M	19,20,21	5.76	0.87	4.96	8.05
	M	22,23,24	5.24	1.46	4.11	5.63
	F	49,50,51	4.58	1.49	4.50	6.59
	F	52,53,54	3.57	2.17	4.52	7.78
G5- Revital-H + Swimming	M	25,26,27	4.29	0.78	4.14	6.42
	M	28,29,30	4.35	0.58	4.54	6.55
	F	55,56,57	2.74	1.62	4.98	7.61
	F	58,59,60	3.18	1.89	4.77	7.99

APPENDIX 7 - INDIVIDUAL ANIMAL SWIMMING TIME OF MALE MICE

Groups	Animal No.	Swimming time (sec)				
		Week 0	Week 1	Week 2	Week 3	Week 4
G2-Swimming control	7	300.00	554.00	416.00	599.00	510.00
	8	342.00	434.00	463.00	666.00	460.00
	9	480.00	333.00	305.00	723.00	529.00
	10	569.00	183.00	1055.00	725.00	470.00
	11	600.00	196.00	930.00	746.00	533.00
	12	840.00	73.00	425.00	480.00	640.00
G3-Amrete 30 ppm + Swimming	13	300.00	816.00	701.00	931.00	595.00
	14	399.00	373.00	524.00	807.00	575.00
	15	445.00	297.00	618.00	529.00	706.00
	16	600.00	240.00	454.00	936.00	621.00
	17	648.00	138.00	297.00	642.00	613.00
	18	900.00	153.00	606.00	916.00	708.00
G4-Amrete 125 ppm + Swimming	19	324.00	231.00	733.00	990.00	705.00
	20	420.00	279.00	918.00	974.00	689.00
	21	454.00	570.00	820.00	960.00	937.00
	22	506.00	357.00	1296.00	1101.00	721.00
	23	660.00	515.00	865.00	867.00	814.00
	24	900.00	564.00	745.00	819.00	913.00
G5-Revital-H + Swimming	25	337.00	670.00	477.00	438.00	940.00
	26	420.00	183.00	413.00	405.00	867.00
	27	480.00	250.00	747.00	675.00	1043.00
	28	525.00	536.00	567.00	1280.00	1257.00
	29	600.00	605.00	627.00	1129.00	1307.00
	30	960.00	93.00	772.00	1125.00	1171.00

APPENDIX 8 - INDIVIDUAL ANIMAL SWIMMING TIME OF FEMALE MICE

Groups	Animal No.	Swimming time (sec)				
		Week 0	Week 1	Week 2	Week 3	Week 4
G2-Swimming control	37	317.00	645.00	800.00	533.00	645.00
	38	369.00	552.00	757.00	491.00	670.00
	39	501.00	511.00	582.00	311.00	699.00
	40	603.00	478.00	707.00	429.00	506.00
	41	630.00	542.00	551.00	366.00	447.00
	42	801.00	752.00	1008.00	313.00	317.00
G3-Amrete 30 ppm + Swimming	43	337.00	356.00	685.00	373.00	778.00
	44	389.00	632.00	458.00	364.00	736.00
	45	431.00	421.00	520.00	309.00	616.00
	46	605.00	297.00	417.00	533.00	670.00
	47	698.00	667.00	727.00	493.00	695.00
	48	816.00	505.00	1136.00	374.00	790.00
G4-Amrete 125 ppm + Swimming	49	350.00	655.00	1043.00	616.00	904.00
	50	400.00	398.00	701.00	372.00	911.00
	51	434.00	297.00	1177.00	548.00	923.00
	52	510.00	332.00	650.00	569.00	837.00
	53	759.00	263.00	573.00	379.00	835.00
	54	843.00	416.00	1106.00	339.00	1087.00
G5-Revital-H + Swimming	55	350.00	571.00	855.00	540.00	1120.00
	56	410.00	436.00	1151.00	505.00	1029.00
	57	448.00	310.00	766.00	541.00	940.00
	58	526.00	289.00	651.00	679.00	1186.00
	59	611.00	653.00	1115.00	683.00	1280.00
	60	854.00	522.00	528.00	661.00	936.00

APPENDIX 9 - INDIVIDUAL ANIMAL CLINICAL CHEMISTRY PARAMETERS OF MALE MICE

Groups	Animal No.	CLINICAL CHEMISTRY PARAMETERS									
		pH	Pco2 mmHg	Po2 mmHg	Chco3 mmol/L	BE(ecf) mmol/L	Na+ mmol/L	K+ mmol/L	Ca++ mmol/L	Cl+ mmol/L	Ctco2 mmol/L
G1-Non Swimming control	1	7.05	70.30	19.90	19.20	-11.30	150.00	5.00	1.19	118.00	21.40
	2	7.03	68.30	27.30	18.00	-12.80	153.00	5.20	1.12	121.00	20.10
	3	6.85	66.20	25.20	11.70	-22.00	147.00	6.80	1.06	119.00	13.70
	4	7.36	21.40	76.20	12.00	-13.40	151.00	2.90	0.78	126.00	12.70
	5	7.08	60.80	20.60	18.10	-11.90	150.00	4.70	1.10	119.00	20.00
	6	6.73	87.70	15.50	11.50	-24.30	153.00	6.60	1.00	126.00	14.20
G2- Swimming control	7	7.09	16.20	75.50	4.90	-24.90	124.00	4.50	0.88	134.00	5.40
	8	7.35	25.00	57.30	13.70	-11.90	148.00	4.80	0.96	121.00	14.50
	9	7.17	25.50	75.90	9.40	-19.10	149.00	3.80	0.94	124.00	10.10
	10	7.14	25.50	76.90	8.80	-20.20	150.00	3.80	0.92	125.00	9.50
	11	6.90	44.00	32.50	8.60	-24.30	150.00	7.00	0.80	129.00	10.00
	12	7.22	32.20	50.50	13.30	-14.40	147.00	3.90	0.62	122.00	14.20
G3-Amrete 30 ppm + Swimming	13	7.11	33.00	76.70	10.50	-19.00	147.00	5.50	1.21	117.00	11.50
	14	7.10	31.90	69.10	10.00	-19.60	148.00	5.20	1.01	122.00	11.00
	15	7.08	37.50	35.70	11.20	-18.70	147.00	5.40	0.72	125.00	12.40
	16	6.89	45.10	44.50	8.60	-24.60	147.00	6.30	0.89	129.00	9.90
	17	7.13	30.80	67.80	10.20	-19.00	150.00	4.00	0.84	125.00	11.10
	18	7.06	26.50	54.00	7.50	-22.80	150.00	6.20	0.85	129.00	8.30
G4-Amrete 125 ppm + Swimming	19	7.31	34.20	54.10	17.00	-9.30	148.00	5.40	1.06	119.00	18.10
	20	7.10	38.20	28.20	11.90	-17.70	147.00	4.20	0.84	120.00	13.10
	21	6.96	52.60	31.60	11.90	-20.10	146.00	6.80	0.82	125.00	13.50
	22	6.97	49.70	33.00	11.50	-20.30	152.00	4.00	0.97	126.00	13.00
	23	7.06	38.50	31.40	10.90	-19.40	149.00	4.90	0.59	126.00	12.10
	24	7.16	43.40	30.30	15.30	-13.50	146.00	4.30	0.78	119.00	16.60
G5- Revital-H + Swimming	25	7.15	38.60	23.40	13.40	-15.40	27.70	4.20	1.01	121.00	9.50
	26	7.14	38.50	22.20	13.20	-15.70	148.00	4.00	0.97	122.00	14.40
	27	7.05	48.50	26.50	13.40	-17.10	149.00	5.50	0.90	124.00	14.90
	28	7.09	29.80	41.60	8.90	-21.00	148.00	6.10	0.75	126.00	9.80
	29	6.92	65.10	26.90	13.30	-19.30	149.00	7.50	1.00	124.00	15.30
	30	7.12	21.30	70.30	6.90	-22.50	152.00	5.00	0.87	128.00	7.60

APPENDIX 9 CONTD., - INDIVIDUAL ANIMAL CLINICAL CHEMISTRY PARAMETERS OF MALE MICE

Groups	Animal No.	CLINICAL CHEMISTRY PARAMETERS						
		Hgb g/dl	Glucose mg/dl	Lactate mmol/L	Creatinine mg/dl	Urea mg/dl	CK U/L	LDH U/L
G1-Non Swimming control	1	11.70	194.00	10.64	0.30	44.69	350.80	920.00
	2	9.90	148.00	11.80	0.30	53.93	163.10	6231.00$
	3	10.70	174.00	14.16	0.92	36.60	3177.50$	1989.00
	4	8.40	83.00	5.06	0.30	35.21	2379.00	1210.00
	5	10.90	169.00	10.14	0.30	52.18	189.20	806.00
	6	10.20	116.00	16.93	0.55	49.88	1103.20	3131.00
G2-Swimming control	7	8.60	82.00	6.93	0.30	88.03	7323.00$	5633.00
	8	10.00	117.00	7.30	0.30	57.32	424.70	4365.00
	9	9.30	130.00	8.74	0.30	41.21	250.30	4809.00
	10	8.80	129.00	8.89	0.30	62.92	551.10	4305.00
	11	6.50	65.00	9.90	0.58	55.36	1796.00	3388.00
	12	7.30	73.00	6.36	0.30	70.50	340.50	1150.00$
G3-Amrete 30 ppm + Swimming	13	12.10	239.00	13.37	0.30	67.10	1310.00	5641.00
	14	11.30	207.00	12.09	0.30	41.02	626.40	5021.00
	15	7.40	62.00	9.30	0.30	52.18	180.70	832.00$
	16	6.90	124.00	11.76	0.30	61.00	1773.60$	5680.00
	17	8.00	109.00	9.32	0.30	53.16	988.10	1204.00
	18	6.90	102.00	10.07	0.30	69.84	496.30	4063.00
G4-Amrete 125 ppm + Swimming	19	10.70	101.00	6.11	0.30	60.01	229.90	1321.00
	20	11.20	59.00	9.65	0.30	67.98	1250.60	3719.00
	21	7.40	37.00	8.74	0..30	62.10	1844.50$	4862.00
	22	10.10	130.00	9.97	0.30	57.62	557.20	6359.00$
	23	6.50	38.00	7.31	0.30	46.09	261.10	1302.00
	24	7.50	79.00	6.51	0.30	58.82	469.00	4189.00
G5-Revital-H + Swimming	25	14.60	140.00	7.29	0.30	64.70	571.20	2473.00
	26	8.70	139.00	7.43	0.30	47.73	521.90	4662.00
	27	8.10	176.00	10.33	0.30	61.60	244.60	2449.00
	28	8.40	45.00	11.01	0.30	68.60	2561.80$	5012.00$
	29	9.90	131.00	14.44	0.68	64.69	657.90	1500.00
	30	7.50	106.00	10.78	0.30	64.67	270..3	3365.00

$ = excluded as significant outlier using Grubbs test

APPENDIX 10 - INDIVIDUAL ANIMAL SERUM BIOCHEMICAL PARAMETERS OF FEMALE MICE

Groups	Animal No.	SERUM BIOCHEMICAL PARAMETERS									
		pH	Pco2 mmHg	Po2 mmHg	Chco3 mmol/L	BE(ecf) mmol/L	Na+ mmol/L	K+ mmol/L	Ca++ mmol/L	Cl+ mmol/L	Ctco2 mmol/L
G1-Non Swimming control	31	7.21	37.50	35.50	15.10	-12.80	153.00	3.80	0.84	124.00	16.20
	32	6.98	65.90	36.90	15.20	-15.90	150.00	5.00	1.16	118.00	17.70
	33	7.24	16.90	79.00	7.20	-20.10	153.00	5.00	0.74	134.00	7.80
	34	7.08	23.20	67.00	6.90	-23.00	152.00	4.60	1.00	126.00	7.60
	35	7.11	19.10	71.90	6.00	-23.60	152.00	4.90	0.73	133.00	6.60
	36	7.01	59.30	47.20	14.90	-16.30	150.00	4.70	1.09	121.00	16.70
G2- Swimming control	37	7.23	19.00	69.80	7.90	-19.70	148.00	3.40	0.87	125.00	8.50
	38	7.32	13.90	61.40	7.20	-18.90	148.00	4.90	0.99	123.00	7.60
	39	7.09	21.40	64.70	6.40	-23.50	150.00	4.00	0.76	130.00	7.10
	40	7.14	19.50	53.30	6.60	-22.40	148.00	4.60	0.94	129.00	7.20
	41	7.01	43.30	28.50	10.90	-20.20	150.00	5.10	0.87	125.00	12.30
	42	7.03	57.50	26.30	15.20	-15.60	153.00	4.20	1.08	123.00	17.00
G3-Amrete 30 ppm + Swimming	43	7.17	21.50	64.50	7.90	-20.50	144.00	4.70	0.93	120.00	8.60
	44	7.18	21.00	66.40	7.90	-20.00	145.00	4.60	0.93	120.00	8.50
	45	7.16	24.90	21.40	8.80	-20.00	146.00	5.60	0.78	123.00	9.60
	46	7.02	42.60	31.00	10.90	-20.20	152.00	5.30	0.82	127.00	12.20
	47	7.15	20.40	65.60	7.20	-21.60	152.00	3.90	0.88	128.00	7.80
	48	7.00	41.20	26.10	10.10	-21.20	150.00	4.30	0.90	124.00	11.40
G4-Amrete 125 ppm + Swimming	49	7.14	19.10	63.80	6.60	-22.40	151.00	5.40	1.02	129.00	7.20
	50	7.22	9.40	54.70	3.90	-23.80	148.00	3.80	0.47	133.00	4.20
	51	7.11	50.70	23.70	16.20	-13.30	152.00	4.40	1.10	122.00	17.70
	52	7.26	15.50	68.70	6.90	-20.30	150.00	3.20	0.90	124.00	7.30
	53	7.23	12.00	78.00	5.10	-22.40	150.00	2.70	0.46	136.00	5.40
	54	7.32	24.20	63.60	12.40	-13.70	148.00	4.30	0.93	123.00	13.20
G5- Revital-H + Swimming	55	7.25	14.90	70.40	6.50	-20.80	145.00	4.20	0.68	125.00	7.00
	56	7.12	26.80	27.50	8.60	-20.80	151.00	5.30	0.89	129.00	9.40
	57	7.16	19.10	58.00	6.90	-21.80	152.00	4.30	0.85	129.00	7.40
	58	7.01	13.60	67.80	4.20	-26.90	152.00	5.60	0.89	131.00	4.70
	59	7.12	15.60	65.70	5.10	-24.20	149.00	5.90	0.78	129.00	5.60
	60	7.06	27.30	45.40	7.70	-22.70	150.00	5.20	0.61	131.00	8.50

APPENDIX 10 CONTD., - INDIVIDUAL ANIMAL SERUM BIOCHEMICAL PARAMETERS OF FEMALE MICE

Groups	Animal No.	SERUM BIOCHEMICAL PARAMETERS						
		Hgb g/dl	Glucose mg/dl	Lactate mmol/L	Creatinine mg/dl	Urea mg/dl	CK U/L	LDH U/L
G1-Non Swimming control	31	10.60	131.00	7.43	0.30	47.88	1308.10$	2199.00
	32	11.20	163.00	13.74	0.37	57.60	199.60	5237.00$
	33	6.30	74.00	9.32	0.30	70.62	618.20	1763.00
	34	8.70	152.00	13.31	0.34	45.59	650.80	1732.00
	35	6.10	74.00	8.98	0.30	47.91	248.50	1232.00
	36	9.10	139.00	12.54	0.31	44.15	535.70	1529.00
G2-Swimming control	37	8.00	103.00	7.64	0.30	53.96	352.90	3917.00$
	38	9.00	183.00	8.66	0.30	59.17	204.50	713.00
	39	6.70	131.00	8.44	0.30	47.17	429.20	1139.00
	40	6.80	141.00	7.62	0.30	53.99	820.30	970.00
	41	8.20	102.00	11.82	0.37	91.95	982.00$	944.00
	42	9.00	135.00	12.56	0.30	65.62	713.50	1375.00
G3-Amrete 30 ppm + Swimming	43	8.10	134.00	9.24	0.34	55.61	367.60	2089.00
	44	7.90	130.00	8.79	0.30	80.47	553.50	3071.00
	45	7.10	91.00	9.50	0.30	80.34	461.70	4570.00$
	46	8.40	58.00	8.75	0.30	67.61	1016.00$	1013.00
	47	7.40	84.00	10.54	0.30	58.40	669.90	945.00
	48	7.90	150.00	13.31	0.35	68.97	344.30	1164.00
G4-Amrete 125 ppm + Swimming	49	10.40	110.00	11.08	0.30	69.41	150.50$	1046.00
	50	3.30	49.00	5.18	0.30	58.38	503.40	1088.00
	51	8.80	145.00	10.19	0.31	42.15	232.80	1046.00
	52	8.10	161.00	9.02	0.30	53.66	587.40	1150.00
	53	3.30	53.00	4.26	0.30	73.80	553.50	804.00
	54	6.90	136.00	7.10	0.30	70.70	282.90	757.00$
G5-Revital-H + Swimming	55	5.30	45.00	5.83	0.30	75.15	641.50	2400.00
	56	7.00	124.00	9.42	0.32	72.09	825.50$	5285.00$
	57	7.90	120.00	9.57	0.30	57.93	327.70	1135.00
	58	7.70	120.00	13.15	0.30	68.37	381.40	1206.00
	59	6.40	98.00	9.98	0.30	60.21	600.50	2882.00
	60	4.50	51.00	8.06	0.36	63.10	644.70	1026.00

$ = excluded as significant outlier using Grubbs test

APPENDIX 11 - INDIVIDUAL ANIMAL GLYCOGEN AND ATP CONCENTRATION IN LIVER AND MUSCLE

Groups	Male				Female			
	Animal No.	Liver Glycogen (µg/mg)	Muscle Glycogen (µg/mg)	Muscle ATP (nmol/mg)	Animal No.	Liver Glycogen (µg/mg)	Muscle Glycogen (µg/mg)	Muscle ATP (nmol/mg)
G1-Non Swimming control	1	13.00	3.62	0.66	31	12.91	3.61	1.05
	2	13.45	3.45	0.87	32	13.21	3.42	0.84
	3	13.45	3.41	0.89	33	12.63	3.21	0.86
	4	13.16	3.70	0.87	34	12.51	3.03	1.05
	5	12.95	3.60	0.87	35	12.58	3.52	0.95
	6	13.88	3.19	0.66	36	12.58	3.31	1.07
G2- Swimming control	7	10.67	2.85	0.68	37	10.52	3.04	0.65
	8	10.84	2.89	0.62	38	10.13	2.95	0.74
	9	11.47	2.61	0.53	39	10.83	2.76	0.65
	10	10.73	2.83	0.68	40	10.62	2.77	0.71
	11	12.80	2.77	0.64	41	11.79	2.81	0.65
	12	10.45	2.67	0.64	42	10.55	3.21	1.07
G3- Amrete 30 ppm + Swimming	13	15.32	5.37	1.06	43	14.74	3.84	1.28
	14	15.23	5.05	1.13	44	13.91	4.59	1.26
	15	15.96	4.44	1.40	45	14.25	4.20	1.03
	16	14.61	5.67	1.06	46	14.13	4.01	1.32
	17	14.22	5.20	1.08	47	12.61	4.35	0.97
	18	14.44	5.53	1.30	48	13.24	5.06	1.30
G4- Amrete 125 ppm + Swimming	19	17.38	7.84	1.30	49	16.41	7.38	1.81
	20	17.01	7.80	1.74	50	15.84	7.21	1.62
	21	16.45	7.51	1.72	51	16.34	7.65	1.85
	22	18.18	7.63	1.49	52	16.44	7.19	1.87
	23	17.17	7.16	1.53	53	16.11	7.00	1.64
	24	16.68	7.65	1.70	54	15.84	7.59	1.51
G5- Revital-H + Swimming	25	19.23	10.17	2.12	55	18.64	8.83	1.91
	26	19.89	10.33	2.12	56	18.64	9.62	2.04
	27	20.10	10.01	2.17	57	18.16	9.70	2.10
	28	19.79	10.33	2.12	58	18.44	9.55	2.12
	29	18.83	10.20	2.14	59	18.70	9.40	1.91
	30	18.88	10.73	2.17	60	18.19	9.60	2.46

APPENDIX 12 - INDIVIDUAL ANIMAL NECROPSY OBSERVATION

Groups	INDIVIDUAL ANIMAL NECROPSY OBSERVATION					
	Male			Female		
	Animal Number	External	Internal	Animal Number	External	Internal
G1-Non Swimming control	1	NAD	NAD	31	NAD	NAD
	2	NAD	NAD	32	NAD	NAD
	3	NAD	NAD	33	NAD	NAD
	4	NAD	NAD	34	NAD	NAD
	5	NAD	NAD	35	NAD	NAD
	6	NAD	NAD	36	NAD	NAD
G2-Swimming control	7	NAD	NAD	37	NAD	NAD
	8	NAD	NAD	38	NAD	NAD
	9	NAD	NAD	39	NAD	NAD
	10	NAD	NAD	40	NAD	NAD
	11	NAD	NAD	41	NAD	NAD
	12	NAD	NAD	42	NAD	NAD
G3-Amrete 30 ppm + Swimming	13	NAD	NAD	43	NAD	NAD
	14	NAD	NAD	44	NAD	NAD
	15	NAD	NAD	45	NAD	NAD
	16	NAD	NAD	46	NAD	NAD
	17	NAD	NAD	47	NAD	NAD
	18	NAD	NAD	48	NAD	NAD
G4-Amrete 125 ppm + Swimming	19	NAD	NAD	49	NAD	NAD
	20	NAD	NAD	50	NAD	NAD
	21	NAD	NAD	51	NAD	NAD
	22	NAD	NAD	52	NAD	NAD
	23	NAD	NAD	53	NAD	NAD
	24	NAD	NAD	54	NAD	NAD
G5-Revital-H + Swimming	25	NAD	NAD	55	NAD	NAD
	26	NAD	NAD	56	NAD	NAD
	27	NAD	NAD	57	NAD	NAD
	28	NAD	NAD	58	NAD	NAD
	29	NAD	NAD	59	NAD	NAD
	30	NAD	NAD	60	NAD	NAD

11. ANNEXURE

ANNEXURE 1 – AAALAC CERTIFICATE

5205 Chairman's Court, Suite 300
Frederick, MD USA 21703

July 10, 2017

Umesh Devurkar, M.V.Sc., Ph.D.
Director and Chairman, IAEC
Management
Vipragen Biosciences Private Limited
No. 67B, Hootagalli Industrial Area
Hebbal
Mysore, Karnataka 570018
India

Dear Dr. Devurkar:

The AAALAC International Council on Accreditation has reviewed the report of the recent site visit to Vipragen Biosciences Private Limited, Mysore, Karnataka, India. The Council commends you and the staff for providing and maintaining a high quality program of laboratory animal care and use. Especially noteworthy were the strong administrative support and commitment to the animal care and use program, evidenced in part by the overall highly qualified staff and the positive interaction of staff at all levels of the institution with the site visitors; the well documented animal facility standard operating procedures (SOP) and record keeping; the dedicated and experienced staff on the animal facility management teams; the good health of all animals; and the clean and well maintained animal facilities. The Council is pleased to inform you that the program conforms with AAALAC International standards as set forth by the *Guide for the Care and Use of Laboratory Animals*, NRC 2011 and guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals. Therefore, **FULL ACCREDITATION** shall continue.

Council acknowledges receipt of the correspondence dated May 6 and March 24, 2017 detailing actions taken relative to concerns expressed by the site visitors during the exit briefing. Specifically, the items addressed satisfactorily included: installing and providing training on a flow regulator for the carbon dioxide euthanasia device; enhancing veterinary clinical care for rabbits; revising sections of the Program Description to reflect accurate information; improving the respiratory protection program; ensuring routine monitoring of rodent bait traps; updating the organizational chart; calibrating the isoflurance vaporizer machine; replacing expired first aid kits; covering lighting fixtures; labeling the feed bins and reverse osmosis water containers with a start date and expiration date; revising the health monitoring program SOP to include long-term animal studies; and ensuring proper humidity control in the bedding storage room.

As stated in the pre-site visit materials, comments and views expressed by site visitors are independent, preliminary opinions and may not necessarily reflect the final peer reviewed judgment of the AAALAC International Council. Please note that the Council discussed the suggestion for improvement (SFI) regarding the use of toxic bait traps for wild rodents used on the outside perimeter of the animal facilities and checked monthly by the contractor. After further deliberation, Council requested that the SFI be retracted. Also, please note that Council discussed the recommendation regarding veterinary clinical care, specifically related to long, sharp nails of rabbits, as a mandatory deficiency; however, after further deliberation, Council considered it to be a SFI.

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