# Histo-Physiological Study of some Parts of Organs Treated with Somadril Drug

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**Abstract:**

Study was conducted to determine the effects of Somadril (carisoprodol) on some hematological, biochemical parameters, and the histological structure in testis of treated rats. Three groups of 5 male rats received 0, 100, 200 mg Somadril per kilogram body weight in diet for thirty days.

Results showed an overall decrease in total leukocyte counts as well as the individual percentage of lymphocytes, and increase in granulocytes when treated by (200mg/kg) of Somadril in comparison to the control group and as well as, the treatment with 100mg/kg of Somadril showed a significant decrease in the individual percentage of granulocytes. Somadril at 100 and 200mg/kg significantly increased erythrocyte count, hemoglobin concentration, and mean corpuscular hemoglobin concentration (MCHC) and the 200mg/kg dose significantly decreased mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH). Additionally, both doses of Somadril caused a significant increase in most measured serum biochemical variables: alanine transaminase (ALT), total bilirubin, creatinine and urea except for the aspartate aminotransferase (AST) and alkaline phosphatase (ALP) whose concentrations showed a significant decrease. Histological examinations in treated rat with Somadril showed testicular vacuolation and degeneration. In addition, decreasing in spermatozoa within the lumen and loss in normal architecture of seminiferous tubules.

**Key words:** Somadril, liver function enzyme, hematology parameters.

## Introduction

The active component of Somadril is carisoprodol, entered the global market more than 50 years ago as a muscle relaxant (1). Carisoprodol is a centrally acting muscle relaxant prescribed for a variety of muscle tension problems, but mostly for lower back pain (2). Animal studies have suggested that carisoprodol metabolizes into

three primary metabolites by hepatic biotransformation: hydroxycarisoprodol, hydroxymeprobamate and meprobamate (3), with meprobamate being the primary active metabolite (4), which are excreted via renal and non-renal pathways (5). While conversion to meprobamate likely contributes to the therapeutic and illicit effects of carisoprodol, the pharmacological and physiological profiles of carisoprodol are not entirely consistent with that of its metabolite, supporting the possibility that carisoprodol may have effects independent of meprobamate (6). Both of carisoprodol and meprobamate are indirect GABAA receptor agonists which open neuronal chloride channels and causes hyperpolarization and the onset of action is 30 minutes with duration of 4 to 6 hours (7).

Other studies reported that Carisoprodol may also adversely affect cardiovascular (tachycardia, postural hypotension and facial flushing), gastrointestinal (nausea, vomiting, hiccup and epigastric distress), and hematologic systems (8). According to Bramness et al. (2007) and Bramness et al. (2008) they were recorded the role of pharmacoepidemiological studies on carisoprodol providing evidence for the risk of psychomotor impairment and traffic accidents, intoxications and abuse (9, 10).

Three month repeated oral dose studies in mice and rats identified the liver and kidney as target organs. High doses caused increased liver weights with minimal to mild centrilobular hypertrophy, probably due to induction of metabolizing enzymes. Increased kidney weights and nephropathy in male and female rats were also observed (11). In another study, Topham et al. (1972) observed that doses of 200 mg/kg carisoprodol to male Wistar-derived Alderley Park rats caused increases in the hepatic enzymes of the microsomal NADPH-electron transport chain; in the male rats, no increases in liver weights were observed (12). Both of the 1988 and 2000 NTP general toxicology studies found that carisoprodol treatment at 1200 mg/kg for 3 months resulted in reduced testes weight and reduced sperm motility in $B6C3F_1$ mice compared to controls, but not in rats. There were no indicators of reproductive toxicity in female reproductive organs (13, 14).

The purpose of the present study was to evaluate the effects of the oral administration of Somadril (carisoprodol) on some hematological indices, some biochemical indices of liver and kidney function tests in the male albino rats, in addition to the examination of some histological sections in testis with sperm morphology compare to the controls.

## Materials and Methods

### Experimental Animals

Adult male albino rats (*Rattus norvegicus*) were obtained from animal house of the department of biology, college of education, Salahaddin University-Erbil. In this study, 15 healthy rats 200-250g weight were used and maintained in controlled conditions of light (12 hrs. light, 12 hrs. dark) and temperature (22° ± 2°C) in an air-conditioned room (15).

### Experimental Design

In this experiment, the total of 15 rats (n = 5) were used and they were fed with standard diet and allowed to drink water ad libitum. The animals were grouped randomly into three groups as followings: Group I (control group): rats of this group received no induction and treatment, group II (T1) treated with 100 mg/kg body weight of Somadril added to the diet, and group III (T2) treated with 200 mg/kg body weight of Somadril added to the diet. The duration of the study was thirty days for each group from the period of November to December, 2013.

### Collection of blood samples

All rats were fasted overnight and then the rats were sacrificed by overdose of ketamine (100 mg/mL) and xylazine (100 mg/mL) in a ratio of 4:1 (v/v) intramuscularly (16). Blood samples were taken from the rats by heart puncture into plastic tubes. After centrifugation (3000 rpm, 10 minutes), sera were separated for biochemical assays using diagnostic kits, and some of blood were added to disposable plastic container contained ethylene diamine tetra acetic acid (EDTA-K3), (4.5mM) as anticoagulants for blood cellular studies using a Coulter Counter (Coulter Electronics, Luton, UK).

### Histological examination

At the end of 30 days of experiment, all animals were dissected then the testis were removed, washed and fixed in10% formalin fixative solution. Subsequently, the tissue was processed (dehydrated, cleaned and then infiltrated) automatically using Automated Tissue Processing Leica (TP1020). Then, the tissues were embedded in paraffin wax using Leica HISTOEMBEDDER. The embedded tissues were sectioned with microtome to produce 5 µm paraffin wax tissue sections. The sections were stained with hematoxylin and eosin followed by mounting with DPX mounting media. Next, the mounted sections were evaluated for microscopic examination using light

microscope (Am Scoop microscope eyepiece camera, China), (17). On the other hand, the epididymis separated and embedded in normal saline for sperm morphology examination.

**Statistical analysis**

The data were expressed as means ± standard error (SE) and statistical analysis was carried out by using completely randomized design (C.R.D.), with five replications. Comparisons between means were made using least significant difference test (L.S.D.) at 5% probability. The statistical analysis was carried out using statgraphics program (18).

**Results and discussion**

**Hematological effects (Table 1)**

T2 group showed an overall decrease in total leukocyte counts as well as the individual percentage of lymphocytes, and increase in granulocytes in comparison to the control group. On the other hand, T1 group showed a significant decrease in the individual percentage of granulocytes. The monocytes did not show much change in the cell counts. Consistent with our results, Ward and Meecham (19) reported the agranulocytosis (leukopenia) occurred following the use of meprobamate. The decrease in circulating leukocytes and neutrophilic granulocytes could be influenced by an alteration of their progenitor cells in bone marrow (20).

Somadril at both doses significantly increased erythrocyte count, hemoglobin concentration, and MCHC and the 200 mg dose significantly decreased MCV and MCH. However, present study showed that the value of hematocrit in treatment with 100mg/kg and 200mg/kg Somadril were unchanged when compared to the control group. Contrary to our results, Chan (14) reported minimal decrease of erythrocyte count, hemoglobin concentration, and MCHC and minimal increase of MCV and MCH as the result of 100 and 200mg/kg carisoprodol administration in rats for 21 days. The decreased MCV and MCH values were accompanied by a minimal increase of erythrocyte (RBC) count; this suggests that, while there were more numbers of circulating RBCs, the red cells were slightly smaller. This study showed significant increased $P<0.05$ in platelet count in the treated groups of male rats with Somadril compare with the control group of male rats. Consistent with our results, Chan (14) reported that administration of carisoprodol along 21 days showed elevation of platelet count if compared to control.

**Biochemical effects (Table 2)**

The daily treated with 100 and 200 mg/kg body weight of Somadril for 30 days produced a significant changes of serum enzymes of the liver function: ALT increased while both AST and ALP decreases, and elevated kidney function variables including urea and creatinine concentrations. Serum total bilirubin concentration showed significant elevation. There are no adequate prospective studies demonstrating the rates of liver enzymes and kidney function changes on carisoprodol therapy or convincing case reports of clinically apparent liver injury due to carisoprodol. Thus, the hepatotoxic potential of this medication is low. It has been increasingly reported as a substance of abuse, taken in higher than recommended doses.

**Histological findings (Table 3 and Plate 1-8)**

It could be observed that the sections obtained from this study with a dose100mg/kg of Somadril gradually produced an abnormal morphology of the testis while a higher dose of 200mg/kg led to a complete destruction of the histological architecture testis. Somadril had a related manner to a progressive destruction of the seminiferous tubules which contributed to low of sperm count and that's observation were in agreement with Grizzle et al. (21) who reported that testicular spermatid concentration was reduced at all levels of carisoprodol.

Testis section with 100mg/kg treated rats showed vacuolation among spermatogonia growing cells, decreasing mature sperms in the lumen (plate 3, 4) and necrotic spermatogonial cells in addition to absent of mature sperms in the lumen (Table 3), (plate 5), while sections of normal rats testis, revealed normal seminiferous tubules, normal spermatogonia, normal primary spermatocyte and mature sperm in the lumen (Table 3), (plate 1, 2) that may be due to treatment with Somadril associated with significant testicular regression and reduction in serum testosterone or may be led to decreased the plasma follicle-stimulating hormone (FSH) and testosterone, in addition to the most common side effect of carisoprodol and meprobamate are consistent with those of other compounds with sedative hypnotic and central nervous system depressant properties (22).

Each of (Plate 6, 7 and 8) testis of rats treated with 200mg/kg they revealed separation of spermatogonia from basement membrane, thickening in the basement membrane, in addition to the absent of mature sperms in the lumen (Table 3) that's mean completely change of seminiferous tubule architecture.

It can be concluded from the results of this work that high doses of carisoprodol led to abnormal morphology of the testis in male adult rats. Therefore, one can conclude that high doses of carisoprodol could lead to infertility in man (22).

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**Table 1: The effect of Somadril (carisoprodol) drug on hematological parameters in male albino rats**

| Treatments / Parameters | Somadril (mg/Kg/day) | | | L.S.D (0.05) |
|---|---|---|---|---|
| | Control (0) | T1 (100) | T2 (200) | |
| **Leukocyte count (109/L)** | 12.05±0.29 | 10.3±0.46 | 7.18±0.96* | 2.11 |
| **Lymphocytes (%)** | 77.05±1.00 | 80.1±1.20 | 66.24±2.94* | 5.94 |
| **Monocytes (%)** | 10.725±0.47 | 10.88±0.26 | 10.68±2.75 | NS |
| **Granulocytes (%)** | 12.225±0.34 | 9.02±0.44* | 23.08±0.48* | 1.32 |
| **Erythrocytes (1012/L)** | 6.96±0.04 | 7.25±0.08* | 7.7±0.07* | 0.22 |
| **Hemoglobin (g/dl)** | 14.12±0.10 | 14.56±0.14* | 14.74±0.16* | 0.43 |
| **Hematocrit (%)** | 38.06±0.33 | 38.68±0.38 | 38.72±0.43 | NS |

| | | | | |
|---|---|---|---|---|
| **MCV (fl)** | 54.8±0.55 | 53.31±0.31 | 50.62±0.57* | 1.52 |
| **MCH (pg)** | 20.26±0.31 | 20.11±0.37 | 19.28±0.22* | 0.96 |
| **MCHC (g/dl)** | 37.08±0.11 | 37.58±0.15* | 37.9±0.12* | 0.40 |
| **Platelets (109/L)** | 495±9.64 | 517.2±4.63* | 541.2±1.46* | 19.21 |

- Data presented as mean ± SE; NS=Non Significant; n=5 in each group, * *P*<0.05

**Table 2: Mean present values Effect of Somadril (carisoprodol) drug on liver and kidney function tests in male albino rats.**

| Treatments / Parameters | Somadril (mg/Kg/day) | | | L.S.D (0.05) |
|---|---|---|---|---|
| | Control (0) | T1 (100) | T2 (200) | |
| **TSB (mg/dl)** | 0.44±0.02 | 0.52±0.03* | 0.56±0.02* | 0.08 |
| **ALP (IU/L)** | 56±1.34 | 55.4±0.74 | 45.4±1.20* | 3.47 |
| **AST (u/L)** | 163.6±3.23 | 140.6±2.83* | 136.6±5.73* | 12.75 |
| **ALT (u/L)** | 38.8±0.37 | 44.8±0.58* | 46.8±0.58* | 1.60 |
| **B. Urea (mg/dl)** | 39.7±0.58 | 41.4±0.42* | 44.4±0.51* | 1.54 |
| **S. Creatinine (mg/dl)** | 0.314±0.02 | 0.354±0.01 | 0.392±0.02* | 0.07 |

- Data presented as mean ± SE; NS=Non Significant; n=5 in each group, * P<0.05

**Table (3): Mean present values of sperm abnormalities in male carisoprodol (Somadril) drug treated rats.**

| Parameters / Treatments | Mean % of normal sperm | Mean % of abnormalities | | | % of total abnormalities |
|---|---|---|---|---|---|
| | | w/o head | w/o tail | w/o hook | |
| **Control** | 64.6 | 12.8 | 12.8 | 9.8 | 35.4 |
| **T1** | 11.6 | 49.4 | 20.2 | 18.8 | 88.4 |
| **T2** | No result* | No result* | No result* | No result* | No result* |

* Duo too completely absent of mature sperms in the lumen

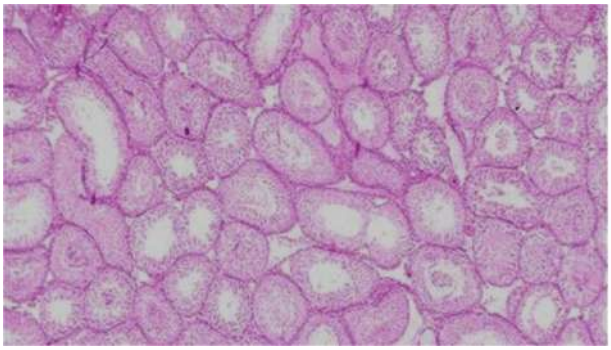

**Plate 1: Histological section of normal rats' testis, revealed normal seminiferous tubules, normal spermatogonia; normal primary**

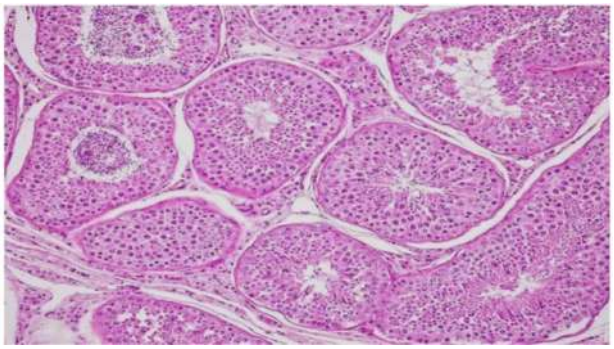

**Plate 2: Histological section of normal rats' testis, revealed normal seminiferous tubules and mature normal sperm in the lumen (H&E**

**spermatocyte and mature normal sperm in the lumen (H&E 40X).**

**100X).**

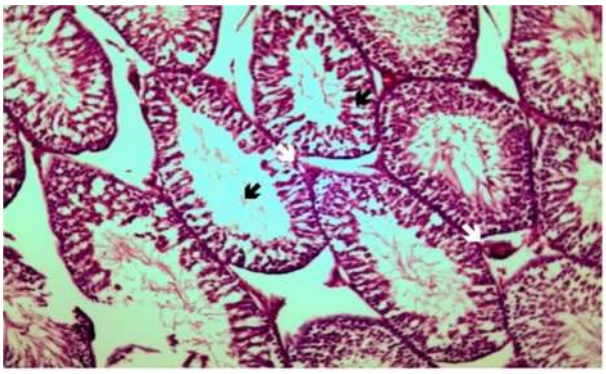

**Plate 3: Histological section of testis of 100 mg/kg group showed vacuolation among spermatogonial growing cells (↘), decreasing in mature sperms in the lumen ( ↙) (H&E 40X).**

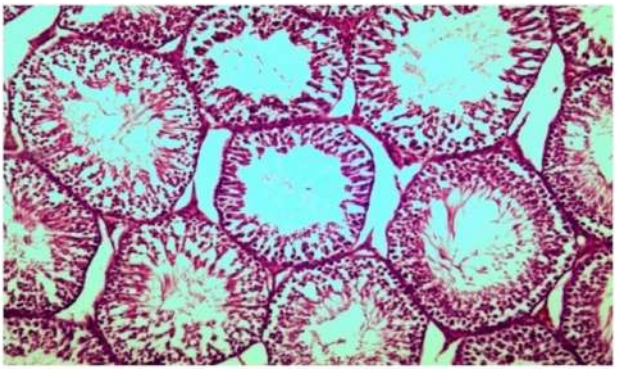

**Plate 4: Histological section of testis of 100 mg/kg group showed vacuolation among spermatogonial growing cells, decreasing in mature sperms in the lumen (H&E 100X).**

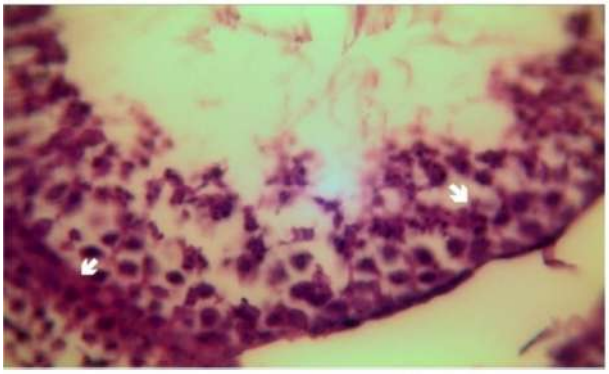

**Plate 5: Histological section of testis of 100 mg/kg group showed vacuolation between spermatogonial growing cells (↘), necrotic spermatogonial cell (↙) in addition to absent of mature sperms in the lumen (H&E 400X).**

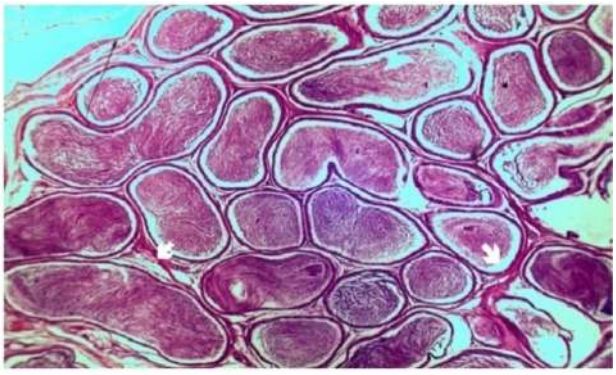

**Plate 6: Histological section of testis of 200 mg/kg group, separation of spermatogonial from basement membrane (↘), thickening in the basement membrane (↙), in addition to the absent of mature sperms in the lumen (H&E 40X).**

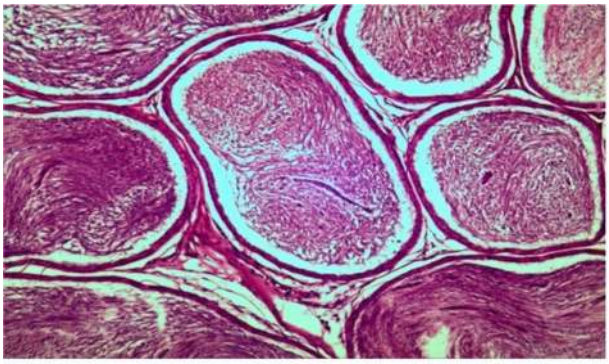

**Plate 7: Histological section of testis of 200mg/kg group, separation of spermatogonial from basement membrane, thickening in the basement membrane, in addition to the absent of mature sperms in the lumen (H&E 100X).**

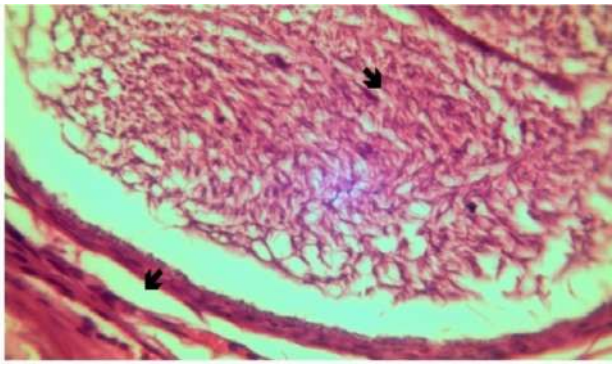

**Plate 8: Histological section of testis of 200mg/kg group, separation of spermatogonial from basement membrane, thickening in the basement membrane (↙), in addition to the absent of mature sperms in the lumen (↘) (H&E 400X).**

## پوختە

ئەم لێکۆڵینەوەیە ئەنجام درا بۆ دیاریکردنی کاریگەری دەرمانی سۆمادریل ﴿کاریزۆپرۆدۆل﴾ لەسەر چەند پارامیتەرێکی خوێن، بایۆکیمیایی، و پێکهاتەی شانەیی گون لە جورجی مامەلەپێکراو. سێ گرووپی ٥ جورجی سۆمادریلی وەرگرت بە 0، 100، 200ملگم بۆ هەر کیلۆیەك لە کێشی لەش لە رێگەی خۆراکەوە بۆ ماوەی سی رۆژ.

ئەنجامەکان دەریانخست کە ژمارەی خرۆکە سپیەکانی خوێن بەشێوەیەکی گشتی و هەروەها ڕێژەی سەدی لیمفەخانەکان دابەزیووە و خانە دەنکۆڵەدارەکان زیادی کردووە کاتێك مامەڵەکراوە بە ﴿200ملگم/ککگم﴾ لە سۆمادریل بە بەراورد بە گروپی کۆنترۆڵ هەروەها، مامەڵەکردن بە ﴿100ملگم﴾ سۆمادریل دابەزینێکی بایەخدار بەدیکرا لە ڕێژەی سەدی خانە دەنکۆڵەدارەکان. سۆمادریل لە دۆزی 100 و 200ملگم/ککگم بوو بەهۆی زیادبونێکی بایەخدار لە ژمارەی خرۆکە سوورەکان، خەستی هیمۆگلۆبین، و ناوەندە خەستی هیمۆگلۆبینی خرۆکەکان ﴿MCHC﴾ هەروەها ژەمەدەرمانی 200ملگم بە شێوەیەکی بایەخدار ناوەندە قەبارەی خرۆکەکان ﴿MCV﴾ و ناوەندە هیمۆگلۆبینی خرۆکەکان ﴿MCH﴾ی کەمکردەوە. سەرباری ئەمەش، هەردوو ژەمەدەرمانی سۆمادریل بوو بەهۆی زیادبوونی بەیەخدار لە زۆربەی گۆڕاوە بایۆکیمیاویەکانی سیرەمی خوێن ﴿ALT، کۆی بیلیرۆبین، کریاتینین و یۆریا﴾ جگە لە ئەنزیمیAST و ALP کە خەستیەکانیان بەشێوەیەکی بایەخدار کەمی کردووە. شیکردنەوەی شانەیی لە جورجە مامەلە پێکراوەکان بە سۆمادریل خراپبوون و بۆشایداربوونی گونی دەرخست. سەرەرای ئەوەش، بووە هۆی کەمبوونەوەی سپڵیرم لەناو بۆشایی و کەمبوونەوە و تێکچوونی شێوەی ئاسایی تۆوە بۆریچکەکان.

## الخلاصة

**أجريت الدراسة لتحديد تأثير السومادريل (كاريزوبرودول) على بعض القياسات الدموية، البيوكيميائية، والتركيب النسيجي في الخصية من الجرذان المعالجة. تلقت ثلاث مجموعات من 5 فئران الذكور 0، 100، 200 ملغ سومادريل لكل كيلوغرام من وزن الجسم في النظام الغذائي لمدة ثلاثين يوما.**

**أظهرت النتائج انخفاضا عاما في إجمالي تعداد الكريات البيض و كذلك نسبة الفردية من الخلايا الليمفاوية، وزيادة في المحببة عندما يعامل من قبل 200ملغم/ كغ) من سومادريل مقارنة مع مجموعة التحكم وكذلك، فإن العلاج (100ملغم) من السومادريل أظهرت انخفاض ملحوظ في نسبة الفردية للخلايا الحبيبيه. سومادريل في 100 ومن 200ملغم/ كغ ادت الى زيادة كبيرة لكريات الدم الحمراء، تركيز الهيموغلوبين، و متوسط تركيز الهيموغلوبين لكرية الدم (MCHC) و جرعة 200ملغم انخفضت بشكل ملحوظ متوسط حجم الكرية الحمراء (MCV) و متوسط هيموغلوبين الكرية (MCH). بالإضافة إلى ذلك، تسببت كلا جرعة من سومادريل زيادة كبيرة في كثير من المتغيرات البيوكيميائية للمصل (ALT، مجموع البيليروبين، الكرياتينين و اليوريا) باستثناء AST وALP التي أظهرت انخفاضا كبيرا. وأظهرت الفحوصات النسيجية في الفئران المعاملة بالسومادريل ظهور فجوات الخصية وانحطاط. وبالإضافة إلى ذلك، تناقص في الحيوانات المنوية داخل تجويف والخسارة في البناء الطبيعي من الأنابيب المنوية.**