A subchronic 90-day oral toxicity study of *Origanum vulgare* essential oil in rats

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Abstract

Oregano essential oil (*Origanum vulgare L. virens*) (OEO) is being used in the food industry due to its useful properties to develop new active packaging systems. In this concern, the safety assessment of this natural extract is of great interest before being commercialized. The European Food Safety Authority requests different *in vivo* assays to ensure the safety of food contact materials. One of these studies is a 90 days repeated-dose oral assay in rodents. In the present work, 40 male and 40 female Wistar rats were orally exposed to 50, 100 and 200 mg/kg body weight (b.w.) OEO during 90 days following the OECD guideline 408. Data revealed no mortality and no treatment-related adverse effects of the OEO in food/water consumption, body weight, haematology, biochemistry, necropsy, organ weight and histopathology. These findings suggest that the oral no-observed-adverse-effect level (NOAEL) of this OEO is 200 mg/kg b.w. in Wistar rats, the highest dose tested. In conclusion, the use of this OEO in food packaging appears to be safe based on the lack of toxicity during the subchronic study at doses 330-fold higher than those expected to be in contact consumers in the worst scenario of exposure.

1. Introduction

Oregano (*Origanum vulgare L.*) is an aromatic plant with a wide distribution throughout the Mediterranean area and Asia (Wei et al., 2016). The composition of oregano essential oil (OEO) commonly includes carvacrol, thymol, α-terpinene and ρ-cymene among other compounds (Burt et al., 2004). The traditional applications of OEO are related to the properties against microorganism and oxidation. Recently, OEO has further application since it is recognised as a natural preservative agent with a strong potential for food preservation (Muriel-Galet et al., 2015). In this sense, the antimicrobial effect of OEO on food has been extensively studied. De Medeiros Barbosa et al. (2016) reported antimicrobial activity of OEO combined with rosemary EO at subinhibitory concentrations in fresh leafy vegetables. Also in meat, OEO has demonstrated to display an antimicrobial effect alone (Soultos et al., 2009; Jayasena and Jo, 2013; Pesavento et al., 2015) and in combination with other EO from clove and cinnamon (Radha krishnan et al., 2014). In addition, OEO has been also useful in cheese not only as antibacterial (Govaris et al., 2011) but also as antioxidant (Asensio et al., 2015). However, the direct addition of OEO may alter the organoleptic characteristics of food and influence negatively in its acceptance. In this sense, Van Haute et al. (2016) reported that the sensorial properties of the meat/fish marinade with OEO and other EOs (thyme and cinnamon) are inevitably affected when the necessary EO concentrations to extend the microbial shelf life are applied. Similarly, a concentration of 4% OEO in active packaging gave rise to unacceptable oregano smell of fresh beef steaks (Camo et al., 2011). Due to the intense aroma of OEO, it can be used in food matrices to provide a balance between sensory acceptability and properties exerted by the spice (Cattelan et al., 2015). In order to avoid the direct incorporation of OEO into food, the active food packaging is a promising trend that allows using OEO that are gradually release from the package to the food (Llana-Ruiz-Cabello et al., 2016a).

The effectiveness of OEO included in food packaging has been confirmed as a preservative in food. Previous experiments carried out in our laboratory have checked the efficacy of polylactic acid films containing 5 and 10% OEO as antioxidant and against microorganism (mainly yeast and molds) in ready-to-eat salads (Llana-Ruiz-Cabello et al., 2016a). A concentration of at least 1% oregano extract in the active packaging system was needed to significantly increase beef display life from 14 to 23 days (Camo et al., 2011). In addition, antioxidant and antimicrobial properties of ethylene vinyl alcohol copolymer films containing OEO and green tea extract components were confirmed (Muriel-Galet et al., 2015).

In fact, OEO containing gelatine films exhibited higher *in vitro* antimicrobial and antioxidant properties than films incorporating lavender EO (Martucci et al., 2015).

Along with the usefulness of OEO, included in active food packaging as preservative, its safety should be also confirmed. In this regard, OEO is categorised as 'generally recognized as safe' (GRAS) by the Food and Drug Administration (Manso et al. 2014) and it is classified as a food additive by the European Union (Muriel-Galet et al., 2015). As flavouring, OEO is normally used in foods at low concentrations. However, the use of these compounds in other applications such as in active packaging may require higher doses that will increase the concern regarding exposure to these compounds (Stammati et al., 1999). In addition, according to the Commission Regulation (EC) No 450/2009, only substances that are included in the Community list of authorised substances may be used in components of active packaging. However, no substance has been included in the list so far. Therefore, a toxicological assessment is needed. The Guidelines of the Scientific Committee on Food for safety assessment of substances used in food contact materials (EFSA, 2016) recommend genotoxicity and subchronic studies in the core set of tests. Genotoxicity studies of OEO and its components are very scarce (EFSA, 2008). However, in the case of the OEO used in the present study the genotoxicity has been evaluated. Results obtained in our laboratory have indicated absence of genotoxic effects of this OEO in rats exposed up to 200 mg/kg body weight (b.w.) (Llana-Ruiz-Cabello et al., 2016c). Carvacrol and thymol, two of the main components of OEO have been also studied. Most of the studies have reported that thymol was neither mutagenic nor genotoxic using *in vitro* assays (Azizan and Blevins, 1995; Stammati et al., 1999; Horvathova et al., 2006; Buyukleyla and Rencuzogullari, 2009; Llana-Ruiz-Cabello et al., 2014a; Maisanaba et al., 2015). However, contradictory results have been obtained for carvacrol using *in vitro* test. It exhibited mutagenic potential and oxidative damage in DNA in the comet assay (Llana-Ruiz-Cabello et al., 2014a). Also, carvacrol showed a weak genotoxic potential on L5178Y/Tk± cells (Maisanaba et al., 2015). Similarly, in mammalian cells, a positive response was obtained in human lymphocytes through the standard comet assay (Aydin et al., 2005a,b). However, other authors have also observed negative results (Stammati et al., 1999; Ündeger et al., 2009; Aydin et al., 2014). In order to complete the toxicological assessment of OEO and its components *in vivo* studies are needed, but those dealing the toxicity of OEO are very scarce. In the case subchronic studies, no previous experiments have been conducted by the moment as far as we know.

Considering all this background, the aim of the present work was to study for the first time the subchronic toxicity of OEO, containing carvacrol/thymol (10:1), intended to be used in active packaging for food applications, in Wistar rats orally exposed to different concentrations of this extract for 90 days. According to the OECD 408 guideline (OECD, 1998), body weight and food and water consumption were recorded. Moreover, clinical observation, haematological and biochemistry parameters, gross and microscopic pathology were performed.

2. Materials and methods

2.1. Supplies and chemicals

Commercial powder neutral gelatine from pork protein (Jesus Navarro S.A., Alicante, Spain) was used as the vehicle for the test item in the dosed groups and control group in the 90-days study. The rest of the chemicals were purchased from Sigma-Aldrich (Madrid, Spain).

2.2. Oregano essential oil analysis

Oregano essential oil was acquired from El Jarpil® (lot number OR2015) (Almería, Spain). It was analysed according to NF ISO 11024 using a Hewlett Packard 5890 chromatograph interfaced to a Hewlett Packard 5970 Mass selective detector (Hewlett Packard, Palo Alto, USA). The gas chromatograph was equipped with a polar column HP INNOWAX, of 60 m x 0.25 mm x 0.5 μm film thickness. The oven temperature was held at 60°C for 6 min, raised to 250°C at 2°C min^{-1}, and maintained at 250°C for 10 min. Helium was used as carrier gas at 22 psi and the injection volume was 1 μL. Compound assignment was achieve by single ion monitoring for various homologous series and via comparison with published and stored data (NKS Library). In the Table 1 the components found in a percentage above 1% have been listed, being the main components carvacrol (55.82%), ρ-cimene (16.39%), thymol (5.14%), γ-terpine (4.71%) and β-cariophyllene (2.40%).

2.3. Diets

In order to select the doses for the 90-day study, the acute oral toxicity study "Up and Down Procedure", OECD 425 (OECD, 2008) was carried out in Wistar rats. No animal died after

dosage up to 2000 mg/kg b.w. of this OEO administered by gavage, so the median lethal dose (LD50) was set above this dose. In addition, a preliminary palatability study evidenced that the maximum dose that was accepted by animals when added to neutral gelatine was 200 mg/kg. Therefore, the selected doses were calculated by dividing this data by a factor of 2, being the doses 50, 100 y 200 mg/kg b.w./day (d). Dietary dose individual formulations were prepared according to Mellado-García et al. (2016).

2.4. Animal and experimental design

The 90-day toxicity study was performed at the Central Service of Experimental Animals from the University of Córdoba (SAE, Córdoba, Spain), in accordance with the OECD Guideline 408 (OECD, 1998), and was codified as 2015-11-SAE. All animals received human care in compliance with the guidelines for the protection of animals used for scientific purposes (Directive 2010/63/EU, Decision 2012/707/UE and RD 53/2013). All procedures have been approved by the Ethical Animal Experimentation Committee of the University of Córdoba, and by the Junta de Andalucía (project no. 05/10/2015-339).

For the 90-day study, 40 male and 40 female Wistar rats, strain Crl. WI (Han) (type outbred rats), were supplied by Charles River Laboratories S.L. (Kings, NY, USA). Rats were fed during the acclimatization period (1 week) with standard laboratory feed (Harlan 2014, Harlan Laboratories, Barcelona, Spain) and water *ad libitum*. During acclimatization, the animals were examined by a veterinary surgeon. At the start of dosing, at approximately 8 weeks of age, average body weight of the males was 297.5±2.0 g and of the females was 188.7±3.6 g.

Animals were individually housed in cages. Rats were randomly assigned to groups (10 rats/sex/group) so that mean body weights were similar for each group. The body weight range did not exceed ±20% of the mean weight/sex at beginning of treatment. The maintenance conditions are described in Mellado-García et al. (2016).

2.5. Body weight, food and water consumption

Rats were observed daily, and body weights, food intake, and water consumption were recorded weekly. The mean body weights per group and sex were calculated weekly from the individual animals. The feed conversion efficiency (FCE) was determined by the ratio of food intake (g)/weight gained (g), according to Escobar et al. (2015).

2.6. Haematology and blood chemistry

Blood samples were collected by intracardiac injection (lightly anesthetized with isoflurane). Animals were fasted overnight prior blood collection (week 13). The haematological parameters studied are described in Mellado-García et al. (2016). Briefly, the haematological parameters evaluated on the automatic hematology analyzer Cell-Dyn 3700 (Abbot, GMI, MI, USA) were: red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MHCH), blood platelet count (PLT), red cell volume distribution (RDW), white blood cell count (WBC), and differential leucocyte count (Neutrophils (NE), Lymphocytes (LY), Monocytes (MO), Eosinophils (EO) and Basophils (BA)).

The standard serum biochemistry parameters were analysed with an automatic chemistry analyzer Cobas 6000 (Roche Diagnostics, IN, USA), to evaluate the following serum biochemistry parameters: glucose (GLUC), blood urea nitrogen (BUN), creatinine (CREAT), bile acids, total cholesterol (CHOL), triglycerides (TRIGL), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALKP), albumin (ALB), total protein (TOT PROT), sodium (Na^+) and potassium (K^+) ions.

2.7. Necropsy, organ weights and histopathology

Rats were fasted overnight (approximately 18 h) and deeply anaesthetized with isofluorane, then euthanized with carbon dioxide inhalation followed by exsanguinated by intracardiac injection. All animals were macroscopically examined as well as the organs after extraction. The following tissues and organs were collected and weighed wet *in situ* after dissection: brain, liver, intestine, stomach, lungs, heart, spleen, kidneys, thymus, adrenal glands, testes and epididims (males), and uterus with cervix and ovaries (females).

According to the guideline OECD 408 (OECD, 1998), full histopathology was carried on the organs and tissues of all animals in the control and the highest dose (200 mg/kg b.w. OEO) groups. Light microscopy examination of liver, kidney, intestine, stomach, lung, heart, and testicle/ovary was performed. Samples were first fixed in 10% buffered formalin for 24 h at 4°C, and then immediately dehydrated in graded series of ethanol, immersed in xylol and embedded in paraffin wax using an automatic processor. Sections of 3-5 mm were mounted.

Afterwards, tissue sections were deparaffinised, rehydrated, stained with haematoxylin and eosin, and mounted with Cristal/Mount (Paraplat, Oxford Labware, St. Louis, MO.).

2.8. Statistical analysis

Continuous variables, including body weight, body weight gain, food and water consumption, haematology, clinical chemistry, and organ weight, were summarized using standard measures of central tendency and dispersion, mean and standard deviation (SD), and were reported by sex and dosage. One-way analysis of variance (ANOVA) was performed to test differences in continuous variables. Normality assumption was tested using Kolmogorov-Sminorv's test. If non-normality, comparison were carried out with Kruskal-Wallis test. If those tests were statistically significant, multiple comparisons were performed using Tukey-Kramer/Dunn's Multiple Comparisons Tests. Differences were considered significant from $P < 0.05$. All statistical analysis were carried out using Graph-Pad Instant software (GraphPadSoftware Inc., La Jolla, USA).

3. Results

3.1. Survival and clinical observations

No mortality occurred during the study. Clinical and ophthalmological examination showed no abnormalities in all groups during the test period. Overall, there were no remarkable treatment-related changes in behaviour or in locomotor activity during the study period. However, one female rat from the control group showed an increase in the scratch pattern, so it was euthanized for humane reasons on day 89. Samples of skin from this animal were analysed and no alteration was observed.

3.2. Body weight, body weight gain, food consumption, feed conversion efficiency, and water consumption

No significant differences were observed in final body weight, body weight gain or total food consumption between rats in any treatment group in comparison to their control groups (Table 2). In addition, the FCE remained unaltered in rats treated with the three doses of OEO.

The mean body weight per week of male and female rats exposed to OEO (50, 100 and 200 mg/kg b.w./d) was not significantly changed through the experiment (Fig. 1). Similarly, no significant changes were observed in % body weight gain in males (Fig. 2a) and females (Fig. 2b). There was no change in food consumption of animals exposed to OEO for 90 days (Supplementary data). Moreover, no consistent differences were recorded in water consumption at any dosage group when compared to the control group (Supplementary data).

3.3. Haematology and blood chemistry

Haematology parameters measured in rats orally exposed to OEO are shown in Table 3. All parameters remained unaltered except the RDW that underwent a significant decrease in females fed with the highest dose (200 mg/kg b.w./d OEO). In addition, no significant differences in total and differential leucocyte counts were recorded for rats fed with OEO in comparison to the untreated male and female rats (Table 4).

Clinical biochemistry parameters of rats subchronically exposed to 50, 100 and 200 mg/kg b.w./d OEO are shown in Table 5. These results revealed only significant differences in GLUC levels in male rats exposed to 50 mg/Kg/d OEO that decreased in comparison to the control group and in female fed with 200 mg/kg b.w./d OEO that increased when compared to the group exposed to 50 mg/Kg/d OEO.

3.4. Necropsy, organ weights and histopathology

Macroscopic examination of organs and tissues from rats subchronically exposed to this OEO revealed no remarkable damage. Moreover, no changes in the organ weight (Table 6) were observed except in kidney in female rats, which weight slightly increased in the group exposed to 200 mg/kg b.w./d OEO in comparison to rats fed with 100 mg/kg b.w./d OEO. Similarly, no significant changes were recorded in the relative organ weight/body weight ratio (Table 7). Overall, no changes were observed in the organ weight/brain weight ratio, although in ovaries it significantly increased only in female rats exposed to 50 mg/kg b.w./d OEO in comparison to the control group (Table 8).

The histological findings described in liver, kidney, intestine, stomach (Fig. 3) and lung, heart and testicle ovary (Fig. 4) of treated rats were similar to those observed in the control groups. No remarkable changes were observed in any organ of male and female rats exposed to 50, 100 and 200 mg/kg b.w./d OEO.

4. Discussion

Food industry is undergoing a renewal to increase the shelf-life of food products by developing new food packaging. New preservative compounds are needed to satisfy consumers' demands. The incorporation of OEO in active packaging is under study (Llana-Ruiz-Cabello et al., 2016a), since it could be a good alternative to synthetic compounds, which have presented undesirable side effects (Branen, 1974; Nerin et al., 2006). However, the safety of OEO needs to be confirmed for this new use in the food industry. In order to accomplish with the requirements suggested by the Scientific Committee on Food for safety assessment of substances used in food contact materials (EFSA, 2016), subchronic studies are needed. A 90-day study in rats with the test material administered via the diet would provide a no-observed-adverse-effect level (NOAEL) (EFSA, 2008). As far as we know, any *in vivo* experiments have studied the subchronic toxicity of OEO so far. However, *in vivo* experimental models have been widely used to confirm the antimicrobial and antioxidant effects of OEO. Thus, OEO dietary supplementation in broilers exhibited a significantly positive effect as growth promoter (Peng et al., 2016). In addition, OEO improved antioxidant activity in a similar way than vitamin E in pigs (Zhang et al., 2015), and even higher effects than vitamin E by improving the pigs' antioxidant status after subchronic exposure (Zou et al., 2015). Moreover, OEO exhibited a protective effect against diquat-induced oxidative injury in intestine of rats (Wei et al., 2016). Ranucci et al. (2015) reported that a combination of OEO and sweet chestnut wood extract may be useful to increase the pig antioxidant status, to prevent lipid oxidation and, thus, to increase meat shelf-life.

Despite no previous *in vivo* studies have been conducted to assess the subchronic effect of OEO, there are several experiments carried out on other EO such as ginger, turmeric, peperina and onion (Liju et al., 2013; Jeena et al., 2014; Escobar et al., 2015; Mellado-García et al., 2016), although they are very scarce. In our work the NOAEL was set at 200 mg/kg b.w./d. Similar NOAEL, 500 mg/kg b.w./d, have been reported for turmeric and ginger EOs, 460 mg/kg b.w./d for peperina EO, and 400 mg/kg b.w./d for the onion OE. Higher NOAELs were found in the case of extracts, for the dry spearmint extract a NOAEL of 1948 mg extract/kg b.w./d was reported on Sprague-Dawley rats (Lasrado et al., 2015), and in a polyphenolic extract of clove buds the NOAEL was 1000 mg/kg b.w./d (Vijayasteltar et al., 2016). In all these studies, no remarkable effects were recorded, only in the case of peperina EO there was an increase in weight gain and FCE in rats fed with approximately 66 mg/kg b.w./d, although it did not follow

a dose-response pattern (Escobar et al., 2015). Likewise, in the present study, the subchronic oral exposure to OEO did not produce any significant adverse effects in the parameters recorded. The subchronic administration of three doses of OEO (50, 100 and 200 mg/kg/d) had negligible effect on haematological parameters. The percentage of RDW was the single parameter undergoing slight decrease, only in one group and in one gender, which can be considered as a marginal change with no biological relevance. Similarly, this parameter was reported to significantly decrease in male and female exposed to mung bean, but the authors stated that there was no biological significance for this observation (Yao et al., 2015). Also, the significant reduction in the RDW of male rats consuming the 7.5% genetically modified (GM) pork diet compared to the group of 7.5% non-GM pork diets were not considered to be adverse (Zhou et al., 2015).

Furthermore, the clinical biochemistry results in the present work only evidenced significant differences in GLUC in male rats exposed to 50 mg/Kg/d OEO that decreased in comparison to the control group and in female fed with 200 mg/Kg/d OEO that increased when compared to the group exposed to 50 mg/Kg/d OEO. Taking into account that these changes did not follow any pattern since they were neither sex-, dose- nor time-dependent they could be considered as not biologically relevant (Pucaj et al., 2011). In fact, the latter authors reported increases in GLUC level in female rats exposed during 90 days to 2.5 mg/kg/d menaquinone-7 and in males fed with the same dose for 44 days; they considered these increases as normal changes. Moreover, GLUC level decreased in male rats fed with 100 mg/kg/d Proallium AP ®, this change being not biologically significant (Mellado-García et al., 2016). However, GLUC changes have been also reported to have biological implications. Hence, male rats fed with conjugated linoleic acid underwent significant reductions in GLUC after 8 and 13 weeks of treated and in female after 4 weeks (O'Hagan and Menzel, 2003). These reductions were related to the influence on insulin level.

Besides, no morphological changes were observed in the present work in any of the tissues studied. As far as we know, this is the first ultrastructural study carried out *in vivo* in this type of EO. However, several authors have described some morphological changes and death cells in different cell lines exposed to OEO from different species. Hence, OEO induced apoptosis in the fibroblasts cell line 5RP7 from the concentration of 125 µg/mL, although this EO was from a different oregano specie *Origanum onites* (Bostancioglu et al., 2012). Similarly, cell death was observed in the intestinal cell line Caco-2 cells exposed up to 500 µg/mL OEO from *Origanum vulgare*, both apoptosis and necrosis, (Dusan et al., 2006; Savini et al., 2009). Considering the concentrations of OEO assayed in the present work *in vivo* are higher than

those used in previous *in vitro* assays, the higher toxicity observed in the *in vitro* studies may be related to other factors. In this regard, the different experimental model, rats in the case of the *in vivo* study and a human cell line in the case of the *in vitro* studies, can influence the results since differences in metabolism and/or in the bioavailability of the OEO may vary substantially (Llana-Ruiz-Cabello et al., 2016a). Moreover, further morphological features were reported when Caco-2 cells were exposed to the main components of OEO, thymol and carvacrol. Thus, thymol induced lipid degeneration, mitochondrial damage, nucleolar segregation as well as apoptosis; and more severe damage was observed for carvacrol such as vacuolated cytoplasm, altered organelles and finally cell death *in vitro* (Llana-Ruiz-Cabello et al., 2014b). In addition, cell death has been reported in the hepatic cell line HepG2, with an increase in the number of necrotic cells after exposure to thymol, while carvacrol caused death cell mainly via apoptosis (Stammati et al., 1999; Yin et al., 2012). Similarly, lung cells A549, breast cancer cells MDA-MB231 and fibroblasts cells 5RP7 treated with carvacrol showed some apoptotic characteristics as well as morphological changes (Koparal and Zeytinoglu, 2003; Arunasree, 2010; Akalin and Incesu, 2011). When comparing the toxicity of OEO and its components, carvacrol and thymol have been reported to induce higher damage than the OEO in HepG2 cells (Özkan and Erdogan, 2011). However, other authors have reported that no single compound seems to be responsible for the cytotoxic effects in Caco-2 cells of the OEO but the whole oil extract (Savini et al., 2009). Overall, many aspects influence the toxicity of OEO such as the concentration assayed, the different composition of each EO and the experimental model used among others.

Provided the NOAEL of the OEO studied in the present work is 200 mg/kg b.w., it is interesting to compare this finding with the real scenario of exposure to consumers. Active packaging containing OEO would be very useful to pack ready-to-eat salads (Llana-Ruiz-Cabello et al., 2016a). In this packaging, 5.5 g of active film is required to pack 1 kg of lettuce. Active film containing 5 and 10% of OEO are both useful as antimicrobial and antioxidant agents (Llana-Ruiz-Cabello et al., 2016a). However, the most likely percentage of OEO to be used in the packaging would be 5% from an economical and safety point of view, since 10% did not increase the effectiveness of the active packaging. Therefore, the maximum concentration that could be released from packaging to lettuce would be 0.27 g OEO per kg. Considering that a medium consumption of lettuce would be 132.53 g per day (http://www.aecosan.msssi.gob.es), the maximum quantity of OEO that could be ingested would be 36.4 mg of OEO per person per day. Considering the worst exposure scenario derived from the results analysed in the present work, an adult consumer weighting 60 kg

would ingest 12,000 mg of OEO per day in the highest dose (200 mg/kg b.w./d); this would be around 330-fold higher than this maximum potential exposure. Therefore, no subchronic toxic effects are expected in the consumption of the OEO used in this study with applications in active food packaging.

5. Conclusions

The present study reveals lack of subchronic toxic effects of OEO given on diet to rats at doses of 50, 100 and 200 mg/kg b.w./d after 90 days. Male and female rats fed with this OEO did not show any change on body weight, food and water consumption, as well as on biochemical and haematological parameters. In addition, normal histopathological features were observed in all tissues. Therefore, the NOAEL obtained in the present work (200 mg/kg b.w./d) is considerable higher than the maximum potential exposure in its use in food packaging.

6. Acknowledgments

Figure Caption

Figure 1. Mean body weights (g) of a) male and b) female rats orally exposed to 50, 100 and 200 mg/kg b.w./d OEO and control rats for 90 days.

Figure 2. Mean body weight gain (%) of a) male and b) female rats orally exposed to 50, 100 and 200 mg/kg b.w./d OEO and control rats for 90-day.

Figure 3. Histopathological analysis of liver, kidney, intestines and stomach of male (A) and female (C) control Wistar rats, and male (B) and female (D) rats subchronically exposed to 200 mg/kg b.w./d OEO for 90-day (bars= 50μm). Normal hepatic cords and normal hepatocytes (He) with polyhedral morphology and rounded central nuclei are observed in liver both in control male and female rats (A,C) and rats exposed to the highest dose of OEO. In kidney, renal parenchyma with normal glomeruli (Gl) and renal tubules are shown for all the groups. Intestine of rats exposed to OEO are observed as intestinal villi (Vi) with normal enterocytes (A,C). Similarly, well developed villi also appear in control groups (B,D). Gastric mucosa with mucosal (Mc) and glandular (Gc) cells apparently normal are shown in all groups.

Figure 4. Histopathological analysis of lung, heart and testicule/ovary of male (A) and female (C) control Wistar rats, and male (B) and female (D) rats subchronically exposed to 200 mg/kg b.w./d OEO for 90-day (bars= 50μm). In lung, normal bronchial epithelia with bronchia (Br) and alveoli (Al) are observed. No remarkable differences were appreciate in cardiac fibers (Fi) of treated (B,D) and untreated groups (A,C). Detail of the testicles of male rats showing normal seminiferous tubules (St) and interstitial space (Is) (A) are observed in both groups (A,B); Ovaries from rats exposed to OEO remained unaltered in comparison to the control rats, both showing normal follicles (F).

Table caption

Table 1. Main components of *Origanum vulgare L.* essential oil.

Table 2. Effect of 90 days oral exposure to OEO on body weight and food consumption in Wistar rats. Values represent the mean ± SD of 10 rats/sex/group. Differences between control and treated groups for male and female rats were evaluated by Kruskal-Wallis test (K.W.) or by ANOVA test (F values).

Table 3. Hematology parameters of Wistar male and female rats fed with 50, 100 and 200 mg/kg b.w./d OEO for 90-days. Values are mean ± SD for 10 rats/sex/group. The differences between control and treated groups for male and female rats were evaluated by Kruskal-Wallis test (K.W.) or by ANOVA test (F values). *p<0.05 in comparison to control group values.

Table 4. Differential white blood cells count data of Wistar male and female rats fed with 50, 100 and 200 mg/kg b.w./d OEO for 90-days. Values are mean ± SD for 10 rats/sex/group. The differences between control and treated groups for male and female rats were evaluated by Kruskal-Wallis test (K.W.) or by ANOVA test (F values).

Table 5. Clinical biochemistry of Wistar male and female rats fed with 50, 100 and 200 mg/kg b.w./d OEO for 90-days. Values are mean ± SD for 10 rats/sex/group. The differences between control and treated groups for male and female rats were evaluated by Kruskal-Wallis test (K.W.) or by ANOVA test (F values). The significance levels observed are *p<0.05 in comparison to control group values, and # p<0.05 when 50 mg/kg/d and 200 mg/kg/d were compared.

Table 6. Absolute organ weight of Wistar male and female rats fed with 50, 100 and 200 mg/kg b.w./d OEO for 90-day. Values are mean ± SD for 10 rats/sex/group. The differences between control and treated groups for male and female rats were evaluated by Kruskal-Wallis test (K.W.) or by ANOVA test (F values). & p<0.05 when 100 mg/kg/d and 200 mg/kg/d were compared.

Table 7. Relative organ weight/body weight of Wistar male and female rats fed with 50, 100 and 200 mg/kg b.w./d OEO for 90-day. Values are mean ± SD for 10 rats/sex/group. The differences between control and treated groups for male and female rats were evaluated by Kruskal-Wallis test (K.W.) or by ANOVA test (F values).

Table 8. Relative organ weight/brain weight of Wistar male and female rats fed with 50, 100 and 200 mg/kg b.w./d OEO for 90-day. Values are mean ± SD for 10 rats/sex/group. The differences between control and treated groups for male and female rats were evaluated by

Kruskal-Wallis test (K.W.) or by ANOVA test (F values). *p<0.05 in comparison to control group values.

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Figure 1

a

Male

b

Female

Figure 2

a **Male**

b **Female**
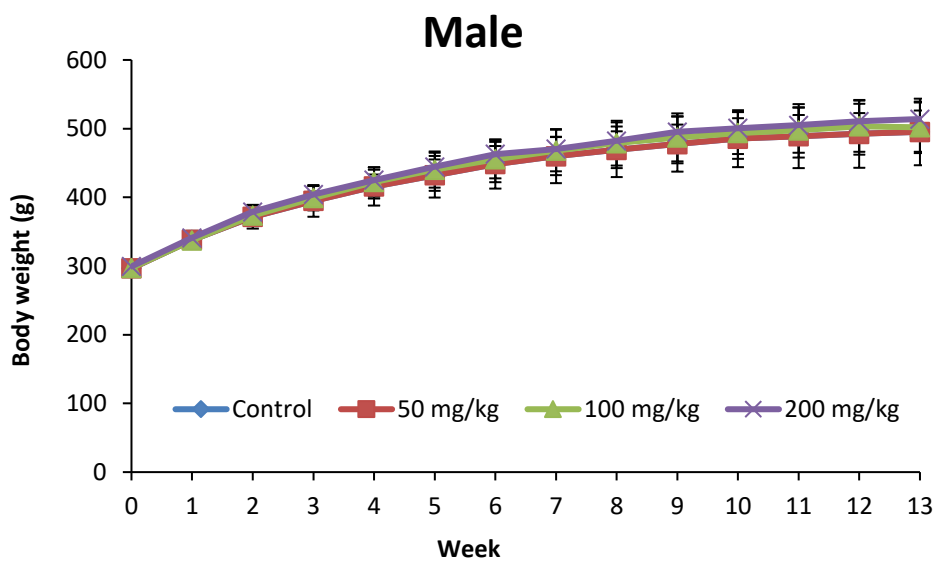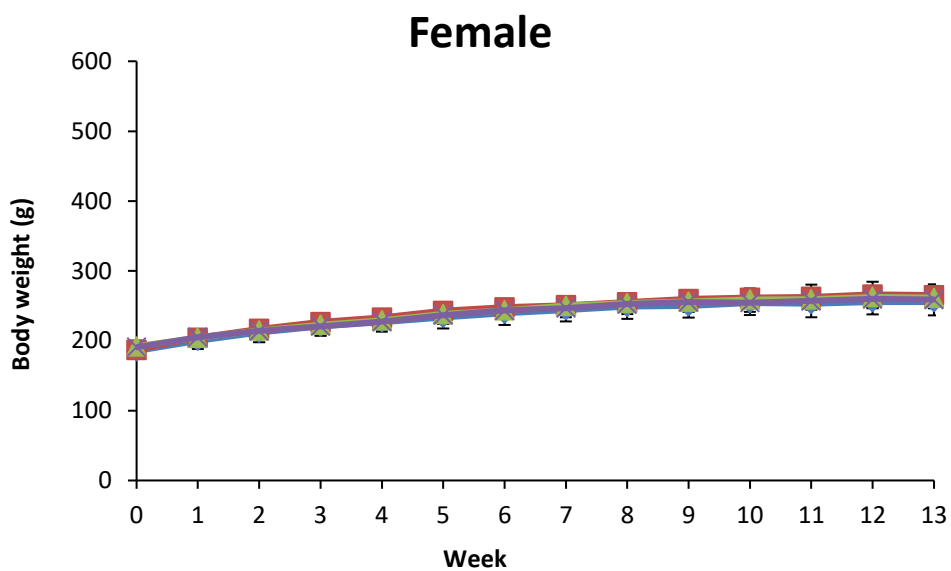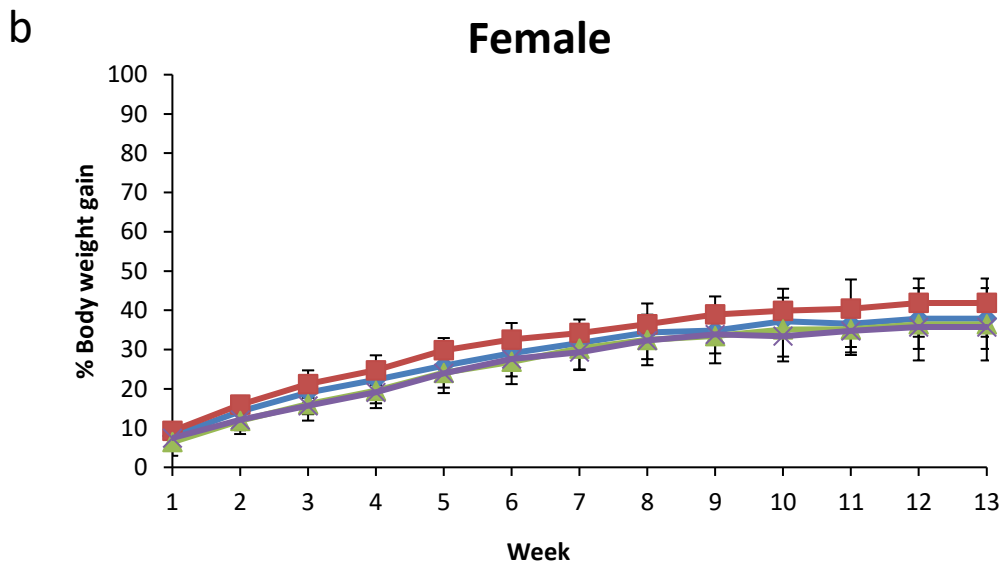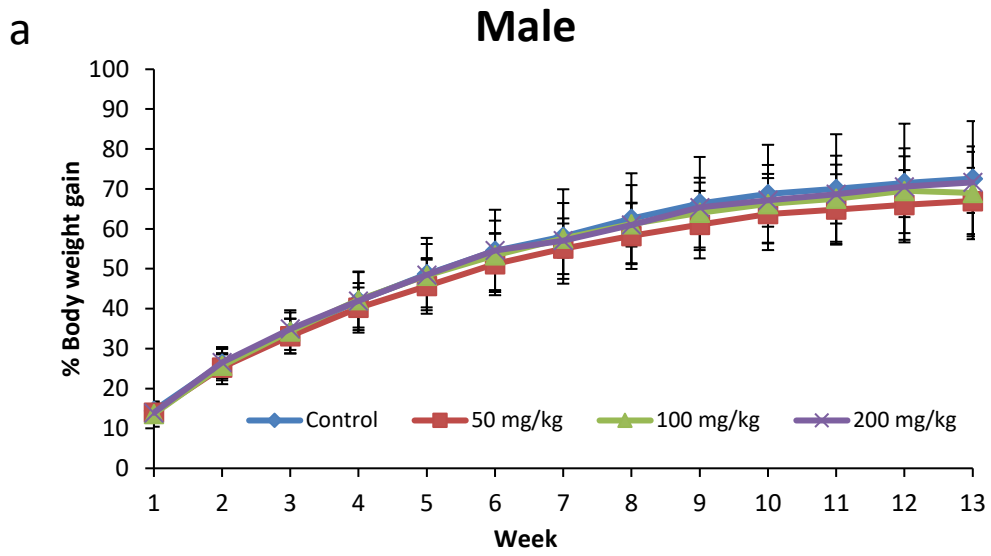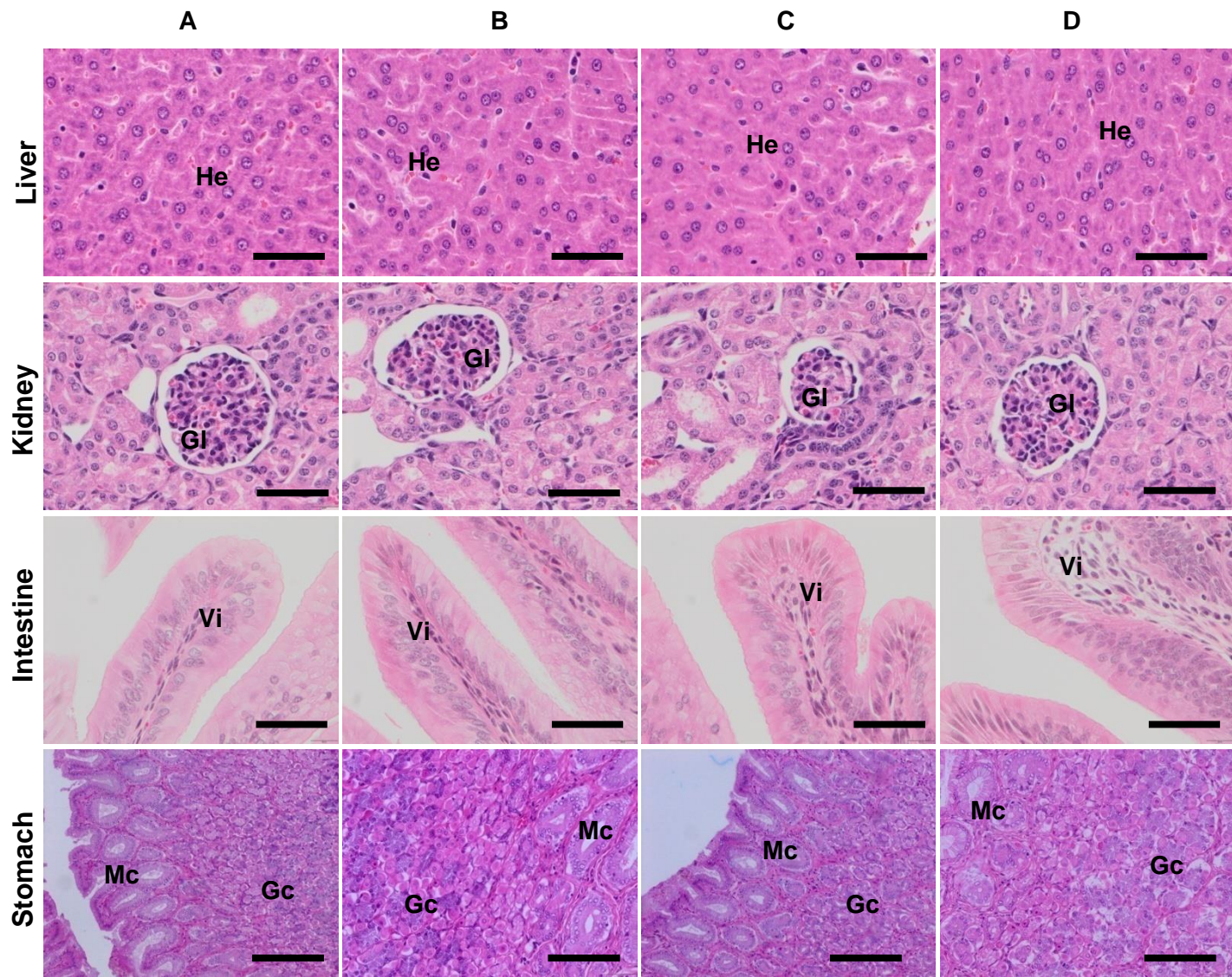
Figure 3

Figure 4
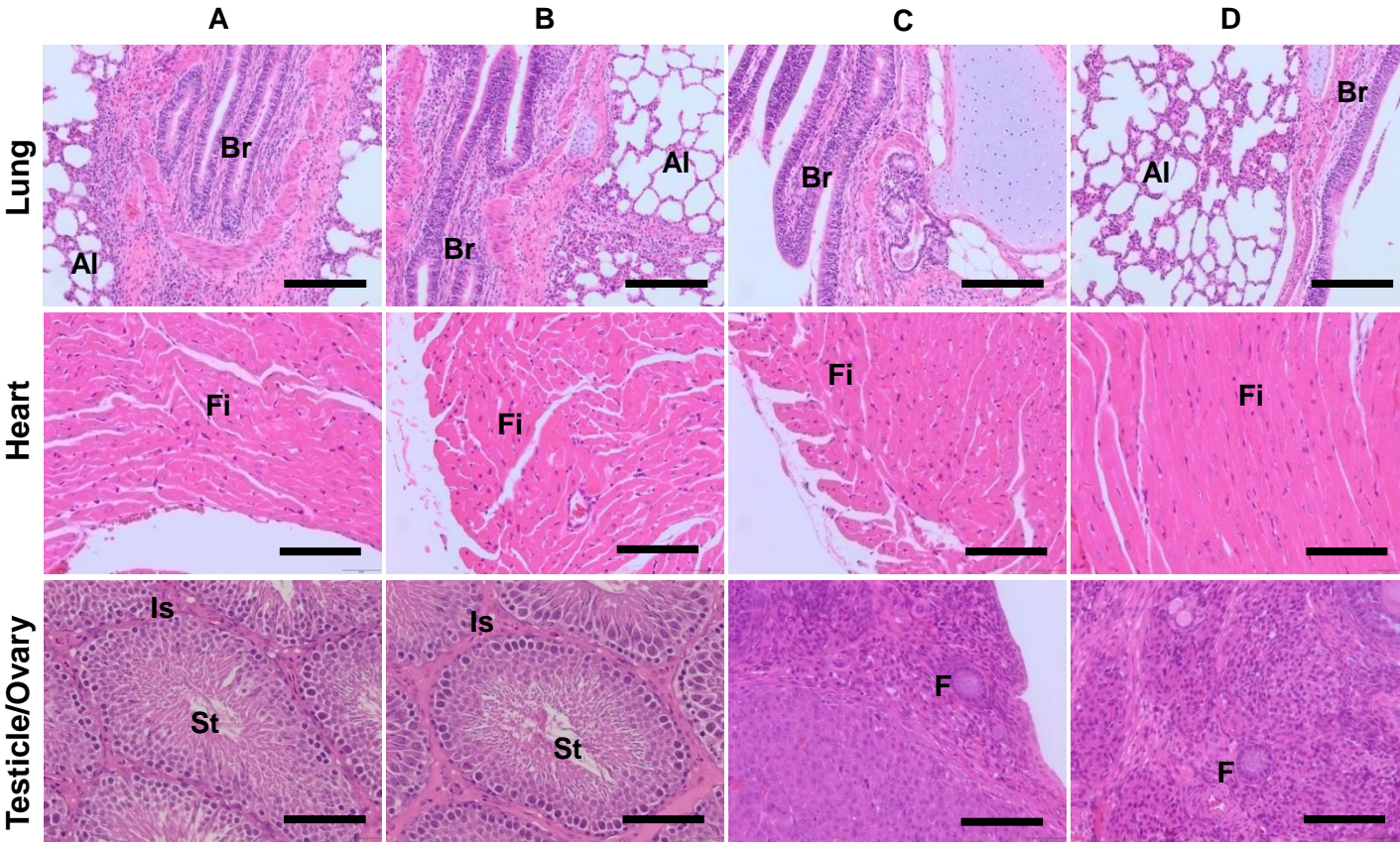
Table 1

RT (min)	Compound	%
10,6	α-PINENE	1,10
10,8	α-THUYENE	1,69
17,9	β-MYRCENE	1,52
19,2	α-TERPINENE	1,62
23,5	γ-TERPINENE	4,71
25,3	p-CYMENE	16,39
36,8	1-OCTEN-3-OL	1,50
47,4	TERPINENE-4-OL	1,33
47,5	β-CARYOPHYLLENE	2,40
79,8	THYMOL	5,14
81,5	CARVACROL	55,82

RT : retention time. Only the main components were reported. (>1%)

Table 2

PARAMETERS	MALE				FEMALE			
	Group 1 (0 mg/Kg/day) N=10	Group 2 (50 mg/Kg/day) N=10	Group 3 (100 mg/Kg/day) N=10	Group 4 (200 mg/Kg/day) N=10	Group 1 (0 mg/Kg/day) N=10	Group 2 (50 mg/Kg/day) N=10	Group 3 (100 mg/Kg/day) N=10	Group 4 (200 mg/Kg/day) N=10
Inicial body weight (g)	296.9±9.7	296.6±5.9	297.1±11.2	299.5±6.1	185.1 ±9.0	187.0 ±5.1	192.1 ±8.0	190.8 ±7.3
	$F_{(36.3)}=0.24$ p=0.88; N.S.				$F_{(36.3)}=1.90$ p=0.42; N.S.			
Final body weight (g)	512.6±48.6	495.4±29.4	502.2±39.7	514.1 ±25.6	255.5 ±19.3	265.4 ±15.2	261.6 ±12.0	258.9 ±12.5
	$F_{(36.3)}=0.58$ p=0.63; N.S.				$F_{(36.3)}=0.77$ p=0.94; N.S.			
Body weight gain	215 ±44.2	198.8 ±26.3	205.1 ±35.7	214.6 ±23.6	70.4±11.4	78.4±12.6	69.5±15.3	68.1±10.2
	$F_{(36.3)}=0.58$ p=0.63; N.S				$F_{(36.3)}=1.38$ p=0.27; N.S.			
Total feed intake (g)	2195.6±159.4	2101.3±168.8	2096.3±138.3	2128.7±93.8	1362.2±120.3	1468.4±128.1	1424.7±92.7	1406.5±61.7
	$F_{(36.3)}=1.02$ p=0.40; N.S.				$F_{(36.3)}=1.79$ p=0.17; N.S.			
Feed conversion ratio	10.5±1.8	10.7±0.9	10.4±1.4	10.0±1.0	19.7±3.0	19.0±2.1	21.2±3.7	21.1±3.6
	$F_{(36.3)}=0.44$ p=0.72; N.S.				$F_{(36.3)}=1.18$ p=0.33; N.S.			

Values are mean ± SD for 10 rats/sex/group.

F: Statistics ANOVA test; K.W: Kruskal-Wallis Statistic; N.S.: Not Significant.

Table 3

		HAEMATOLOGY DATA SUMMARY							
		MALE				FEMALE			
		Group 1 (0 mg/Kg/day) N=10	Group 2 (50 mg/Kg/day) N=10	Group 3 (100 mg/Kg/day) N=10	Group 4 (200 mg/Kg/day) N=10	Group 1 (0 mg/Kg/day) N=10	Group 2 (50 mg/Kg/day) N=10	Group 3 (100 mg/Kg/day) N=10	Group 4 (200 mg/Kg/day) N=10
RBC $10^6/\mu l$	MEAN	9.1	9.3	9.4	9.4	7.8	7.8	8.0	7.8
	ST. DEV.	0.6	0.6	0.5	0.6	0.4	0.8	0.4	0.6
			$F_{(36.3)}=0.4373$ p=0.73; N.S.				$F_{(36.3)}=0.20$ p=0.90; N.S.		
HGB g/dL	MEAN	13.9	13.9	14.4	14.5	12.8	13.4	14.3	13.0
	ST. DEV.	0.9	1.0	1.2	0.6	1.8	1.4	0.8	1.5
			KW= 3.05 p=0.39; N.S.				KW= 4.74 p=0.19; N.S.		
HCT %	MEAN	69.9	70.6	71.9	70.6	65.3	65.4	66.8	64.4
	ST. DEV.	3.0	4.3	3.9	3.0	3.5	5.7	3.2	4.4
			$F_{(36.3)}=0.54$ p=0.66; N.S.				KW= 1.81 p=0.61; N.S.		
MCV fL	MEAN	77.1	75.8	77.0	75.5	83.4	83.6	84.0	83.0
	ST. DEV.	2.1	1.9	2.3	2.0	2.3	2.3	1.9	2.1
			$F_{(36.3)}=1.42$ p=0.25; N.S.				$F_{(36.3)}=0.29$ p=0.83; N.S.		
MCH pg	MEAN	15.4	14.9	15.4	15.5	16.4	17.2	18.0	16.7
	ST. DEV.	1.7	1.5	1.6	1.3	2.1	1.4	0.5	1.2
			KW= 1.50 =0.68; N.S.				$F_{(36.3)}=2.50$ p=0.08; N.S.		
MCHC g/dL	MEAN	20.0	19.6	20.0	20.6	19.6	20.6	19.4	20.1
	ST. DEV.	1.8	2.0	2.1	1.5	2.2	1.6	6.3	1.5
			KW= 2.50 =0.48; N.S.				KW= 2.55 =0.47 ; N.S.		
PLT $10^3/\mu l$	MEAN	883.3	901.5	1217.1	1142.0	597.8	755.4	834.4	829.1
	ST. DEV.	256.3	358.0	403.0	418.9	264.2	207.8	135.5	156.8
			KW= 4.75 =0.19; N.S.				$F_{(36.3)}=2.76$ p=0.06; N.S.		
RDW %	MEAN	16.4	17.1	16.6	16.8	15.5	15.0	15.4	14.3*
	ST. DEV.	1.4	1.3	0.8	1.3	1.2	0.7	0.8	0.8
			KW= 1.86 =0.60; N.S.				KW= 8.78 =0.03; *p<0.05		

RBC: Erythrocyte count; HGB: hemoglobin; HCT: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; PLT: platelet (thrombocyte) count; RDW: red blood cell distribution width; K.W: Kruskal-Wallis Statistic; N.S.: Not Significant.

Table 4

		MALE				FEMALE			
		Group 1	**Group 2**	**Group 3**	**Group 4**	**Group 1**	**Group 2**	**Group 3**	**Group 4**
		(0 mg/Kg/day)	**(50 mg/Kg/day)**	**(100 mg/Kg/day)**	**(200 mg/Kg/day)**	**(0 mg/Kg/day)**	**(50 mg/Kg/day)**	**(100 mg/Kg/day)**	**(200 mg/Kg/day)**
		N=10	**N=10**	**N=10**	**N=10**	**N=10**	**N=10**	**N=10**	**N=10**
WBC	MEAN	8.7	10.0	9.4	8.1	6.6	7.6	8.1	11.3
$10^3/\mu L$	ST. DEV.	3.3	6.3	1.6	1.1	2.7	3.5	2.2	8.3
		KW= 2.43 p=0.49; N.S.				**KW= 2.32 p=0.51; N.S.**			
NE	MEAN	25.4	35.9	33.7	41.9	21.6	23.1	24.8	32.7
%	ST. DEV.	9.2	12.4	12.5	17.8	18.6	13.9	16.3	16.4
		F(36.3)= 2.58 p=0.07; N.S.				**KW= 2.17 p=0.54; N.S.**			
LY	MEAN	65.4	53.8	56.2	48.3	73.5	67.3	68.4	57.1
%	ST. DEV.	11.2	13.4	13.6	16.6	19.6	18.3	16.6	20.7
		KW= 7.01 p=0.07; N.S.				**KW= 2.94 p=0.40; N.S.**			
MO	MEAN	4.6	5.3	5.1	3.6	4.3	4.7	4.3	4.9
%	ST. DEV.	2.2	3.3	1.2	2.2	2.4	2.5	1.9	2.6
		F(36.3)= 0.85 p=0.47; N.S.				**F(36.3)= 0.12 p=0.95; N.S.**			
EO	MEAN	4.3	4.4	4.5	5.6	1.3	4.2	2.0	3.8
%	ST. DEV.	4.0	1.7	2.5	3.1	0.6	5.1	1.0	2.2
		F(36.3)= 0.40 p=0.75; N.S.				**F(36.3)= 2.07 p=0.12; N.S.**			
BA	MEAN	0.4	0.7	0.5	0.6	0.5	0.6	0.5	0.5
%	ST. DEV.	0.2	0.2	0.3	0.2	0.2	0.3	0.3	0.3
		F(36.3)= 1.80 p=0.16; N.S.				**F(36.3)= 0.84 p=0.48; N.S.**			

Title: DIFFERENTIAL WHITE BLOOD CELLS COUNT DATA SUMMARY

WBC: total leukocyte count; NE: neuthrophil; LY: lymphocyte; MO:monocyte; EO:eosinophil; BA:basophil;

F: Statistics ANOVA test; K.W:Kruskal-WallisStatistic; N.S.: Not Significant.

Table 5

		MALE				FEMALE			
CLINICAL BIOCHEMISTRY DATA SUMMARY									
		Group 1 (0 mg/Kg/day) N=10	Group 2 (50 mg/Kg/day) N=10	Group 3 (100 mg/Kg/day) N=10	Group 4 (200 mg/Kg/day) N=10	Group 1 (0 mg/Kg/day) N=10	Group 2 (50 mg/Kg/day) N=10	Group 3 (100 mg/Kg/day) N=10	Group 4 (200 mg/Kg/day) N=10
GLUC tmg/dL	MEAN	143.7	122.8*	126.8	129.3	128.5	123.2	129.0	154.3#
	ST. DEV.	18.1	14.6	11.2	13.3	18.2	17.0	11.2	36.2
		$F_{(36.3)}=3.87$ *$p<0.05$				$F_{(36.3)}=3.79$#$p<0.05$			
UREA mg/dl	MEAN	38.1	38.0	36.5	35.6	41.0	37.7	37.3	36.5
	ST. DEV.	4.2	5.3	6.7	3.8	6.5	5.1	4.3	4.8
		$F_{(36.3)}=0.54$ $p=0.66$; N.S.				$F_{(36.3)}=1.41$ $p=0.25$; N.S.			
CREAT mg/dL	MEAN	0.2	0.3	0.3	0.3	0.3	0.3	0.3	0.3
	ST. DEV.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
		$F_{(36.3)}=2.30$ $p=0.09$; N.S.				$F_{(36.3)}=0.63$ $p=0.60$; N.S.			
Bile acids μMol	MEAN	36.2	31.5	34.2	25.9	54.7	57.0	54.1	44.4
	ST. DEV.	13.3	13.3	12.9	11.2	20.2	30.6	16.3	32.4
		$F_{(36.3)}=1.24$ $p=0.31$; N.S.				KW=1.79 $p=0.62$; N.S.			
CHOL mg/dL	MEAN	64.9	66.3	67.1	60.9	63.6	59.5	68.8	61.2
	ST. DEV.	9.1	9.3	9.8	14.2	12.0	9.8	17.5	8.6
		KW=2.48 $p=0.48$; N.S.				$F_{(36.3)}=1.06$ $p=0.38$; N.S.			
TRIGL mg/dL	MEAN	128.8	100.8	111.8	122.2	63.2	58.1	69.0	66.6
	ST. DEV.	58.6	31.3	32.6	59.2	22.4	12.2	15.9	18.3
		$F_{(36.3)}=0.67$ $p=0.58$; N.S.				$F_{(36.3)}=0.72$ $p=0.54$; N.S.			
AST U/L	MEAN	136.2	125.9	109.8	117.9	139.3	118.3	136.4	138.89
	ST. DEV.	33.4	20.9	19.2	31.8	51.9	23.4	35.5	35.0
		$F_{(36.3)}=1.74$ $p=0.18$; N.S.				$F_{(36.3)}=0.64$ $p=0.60$; N.S.			
ALT U/L	MEAN	31.4	35.6	28.5	34.5	31.9	26.8	36.9	37.3
	ST. DEV.	5.4	8.7	6.3	12.7	18.3	10.4	14.9	14.1
		$F_{(36.3)}=1.33$ $p=0.28$; N.S.				KW=4.36 $p=0.23$; N.S.			
ALKP U/L	MEAN	62.1	60.8	54.8	51.8	24.0	25.6	24.9	19.7
	ST. DEV.	11.7	13.7	8.6	6.7	5.6	6.5	6.1	5.5

		KW=6.10 p=0.11; N.S.				**F(36.3)=2.00 p=0.13; N.S.**			
ALB	MEAN	3.8	3.6	3.6	3.6	3.8	3.7	4.0	3.8
g/dl	ST. DEV.	0.1	0.2	0.2	0.2	0.4	0.4	0.3	0.3
		KW=0.44 p=0.93; N.S.				**KW=2.78 p=0.43; N.S.**			
TOT PROT	MEAN	6.1	6.1	6.1	6.1	5.8	0.7	6.0	5.7
g/dl	ST. DEV.	0.4	0.2	0.2	0.4	0.4	0.4	0.4	0.4
		KW=0.06 p=0.30; N.S.				**F(36.3)=1.09 p=0.36; N.S.**			
Na⁺	MEAN	127.1	127.9	128.6	129.4	126.4	127.8	127.0	126.6
mmol/L	ST. DEV.	3.5	2.5	3.7	3.6	6.1	1.9	2.1	2.6
		F(36.3)=0.84 p=0.48; N.S.				**F(36.3)=0.30 p=0.83; N.S.**			
K⁺	MEAN	8.6	8.6	7.9	7.7	6.6	6.5	6.1	5.8
mmol/L	ST. DEV.	1.3	1.1	0.6	0.6	1.1	1.1	0.9	0.7
		F(36.3)=2.27 p=0.10; N.S.				**F(36.3)=1.42 p=0.25; N.S.**			

GLUC: glucose; BUN: blood urea nitrogen; CREAT: creatinine; Bili-T: Bilirubin, total; CHOL: cholesterol, total; TRIGL: triglycerides; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALKP: alkaline phosphatase; ALB: albumin; TOT PROT: protein, total; Na⁺:sodium; K⁺:potassium.

F: Statistics ANOVA test; K.W: Kruskal-Wallis Statistic; N.S.: Not Significant

Table 6

ORGAN WEIGHT DATA SUMMARY

MALE

		Group 1 (0 mg/Kg/day) N=10	Group 2 (50 mg/Kg/day) N=10	Group 3 (100 mg/Kg/day) N=10	Group 4 (200 mg/Kg/day) N=10
BODY W. (g)	MEAN	495.2	477.1	485.3	495.7
	ST. DEV.	48.6	27.8	36.9	25.8
		$F_{(36.3)}=0.61$ p=0.61; N.S.			
BRAIN (g)	MEAN	2.0	2.0	2.0	2.0
	ST. DEV.	0.2	0.2	0.1	0.1
		$F_{(36.3)}=0.05$ p=0.98; N.S.			
LIVER (g)	MEAN	15.7	14.0	14.4	14.8
	ST. DEV.	2.5	1.4	1.7	1.7
		$F_{(36.3)}=1.49$ p=0.23; N.S.			
HEART (g)	MEAN	1.5	1.4	1.5	1.3
	ST. DEV.	0.2	0.2	0.3	0.1
		$F_{(36.3)}=1.47$ p=0.24; N.S.			
SPLEEN (g)	MEAN	0.7	0.8	0.7	0.8
	ST. DEV.	0.1	0.1	0.1	0.2
		$F_{(36.3)}=0.94$ p=0.43; N.S.			
KIDNEYS (g)	MEAN	3.3	3.2	3.1	3.2
	ST. DEV.	0.4	0.4	0.3	0.2
		$F_{(36.3)}=0.57$ p=0.64; N.S.			
THYMUS (g)	MEAN	0.8	0.7	0.8	0.7
	ST. DEV.	0.2	0.2	0.2	0.2
		$F_{(36.3)}=1.10$ p=0.35; N.S.			
TESTES (g)	MEAN	4.0	3.9	3.9	4.0
	ST. DEV.	0.6	0.4	0.4	0.4
		$F_{(36.3)}=0.42$ p=0.73; N.S.			
EPIDIDIMS (g)	MEAN	2.6	2.3	2.1	2.6
	ST. DEV.	0.7	0.7	0.6	0.5
		$F_{(36.3)}=1.45$ p=0.24; N.S.			
ADRENALS (g)	MEAN	0.2	0.2	0.1	0.2
	ST. DEV.	0.1	0.2	0.1	0.1
		KW=2.10 p=0.56; N.S.			

FEMALE

		Group 1 (0 mg/Kg/day) N=10	Group 2 (50 mg/Kg/day) N=10	Group 3 (100 mg/Kg/day) N=10	Group 4 (200 mg/Kg/day) N=10
BODY W. (g)	MEAN	246.1	255.0	250.7	247.0
	ST. DEV.	16.3	14.4	41.6	12.5
		$F_{(36.3)}=0.82$ p=0.49; N.S.			
BRAIN (g)	MEAN	1.7	1.7	1.7	1.7
	ST. DEV.	0.2	0.2	0.1	0.1
		$F_{(36.3)}=0.77$ p=0.52; N.S.			
LIVER (g)	MEAN	6.3	6.8	6.9	6.8
	ST. DEV.	0.5	0.8	0.7	0.8
		$F_{(36.3)}=1.21$ p=0.32; N.S.			
HEART (g)	MEAN	0.8	0.9	0.9	0.9
	ST. DEV.	0.1	0.1	0.1	0.1
		$F_{(36.3)}=1.85$ p=0.16; N.S.			
SPLEEN (g)	MEAN	0.5	0.5	0.5	0.5
	ST. DEV.	0.0	0.1	0.1	0.1
		KW=0.35 p=0.95; N.S.			
KIDNEYS (g)	MEAN	1.9	2.0	1.9	2.1&
	ST. DEV.	0.2	0.2	0.1	0.2
		$F_{(36.3)}=4.388$ &$p<0.05$			
THYMUS (g)	MEAN	0.5	0.5	0.6	0.6
	ST. DEV.	0.2	0.2	0.2	0.2
		$F_{(36.3)}=0.67$ p=0.58; N.S.			
UTE./CERV. (g)	MEAN	0.9	1.0	0.8	0.7
	ST. DEV.	0.3	0.3	0.2	0.1
		$F_{(36.3)}=2.18$ p=0.11; N.S			
OVARIES (g)	MEAN	0.5	0.7	0.6	
	ST. DEV.	0.0	0.1	0.1	
		KW=6.23 p=0.10; N.S			
ADRENALS (g)	MEAN	0.2	0.2	0.2	0.2
	ST. DEV.	0.1	0.0	0.1	0.1
		KW=1.10 p=0.36; N.S			

F: Statistics ANOVA test; K.W: Kruskal-Wallis Statistic; N.S.: Not Significant

Table 7

ORGAN WEIGHT/BODY WEIGHT RATIO DATA SUMMARY												
MALE						FEMALE						
		Group 1	Group 2	Group 3	Group 4			Group 1	Group 2	Group 3	Group 4
		(0 mg/Kg/day)	(50 mg/Kg/day)	(100 mg/Kg/day)	(200 mg/Kg/day)			(0 mg/Kg/day)	(50 mg/Kg/day)	(100 mg/Kg/day)	(200 mg/Kg/day)
		N=10	N=10	N=10	N=10			N=10	N=10	N=10	N=10
BRAIN (%)	MEAN	0.41	0.41	0.41	0.40	BRAIN (%)	MEAN	0.59	0.59	0.62	0.60
	ST. DEV.	0.06	0.04	0.04	0.04		ST. DEV.	0.13	0.07	0.07	0.06
		KW=0.73 p=0.87; N.S.						F(36.3)=0.29 p=0.84; N.S.			
LIVER (%)	MEAN	3.16	2.93	2.96	2.99	LIVER (%)	MEAN	2.26	2.43	2.51	2.45
	ST. DEV.	0.39	0.25	0.23	0.25		ST. DEV.	0.15	0.27	0.24	0.32
		F(36.3)=1.31 p=0.29; N.S.						F(36.3)=1.81 p=0.16; N.S.			
HEART (%)	MEAN	0.30	0.30	0.30	0.27	HEART (%)	MEAN	0.30	0.34	0.34	0.32
	ST. DEV.	0.04	0.03	0.04	0.02		ST. DEV.	0.03	0.05	0.04	0.05
		F(36.3)=1.97 p=0.14; N.S.						F(36.3)=1.62 p=0.20; N.S.			
SPLEEN (%)	MEAN	0.15	0.16	0.15	0.16	SPLEEN (%)	MEAN	0.18	0.19	0.19	0.17
	ST. DEV.	0.02	0.02	0.02	0.03		ST. DEV.	0.02	0.04	0.04	0.04
		F(36.3)=1.00 p=0.40; N.S.						F(36.3)=0.46 p=0.71; N.S.			
KIDNEYS (%)	MEAN	0.67	0.67	0.65	0.64	KIDNEYS (%)	MEAN	0.68	0.71	0.69	0.77
	ST. DEV.	0.07	0.08	0.05	0.04		ST. DEV.	0.05	0.11	0.07	0.12
		F(36.3)=0.79 p=0.51; N.S.						F(36.3)=1.70 p=0.18; N.S.			
THYMUS (%)	MEAN	0.17	0.14	0.16	0.15	THYMUS (%)	MEAN	0.18	0.19	0.23	0.20
	ST. DEV.	0.05	0.04	0.04	0.05		ST. DEV.	0.07	0.05	0.06	0.06
		F(36.3)=0.86 p=0.47; N.S.						F(36.3)=0.99 p=0.41; N.S.			
TESTES (%)	MEAN	0.82	0.82	0.80	0.82	UTE./CERV. (%)	MEAN	0.33	0.36	0.29	0.27
	ST. DEV.	0.13	0.08	0.08	0.07		ST. DEV.	0.11	0.11	0.09	0.06
		F(36.3)=0.11 p=0.95; N.S.						F(36.3)=1.63 p=0.20; N.S.			
EPIDIDIMS (%)	MEAN	0.53	0.48	0.44	0.52	OVARIES (%)	MEAN	0.19	0.23	0.21	0.19
	ST. DEV.	0.13	0.15	0.15	0.10		ST. DEV.	0.02	0.04	0.04	0.04
		KW=2.84 p=0.42; N.S.						F(36.3)=2.90 p=0.05; N.S.			
ADRENALS (%)	MEAN	0.04	0.05	0.03	0.04	ADRENALS (%)	MEAN	0.07	0.07	0.08	0.08
	ST. DEV.	0.02	0.04	0.02	0.01		ST. DEV.	0.02	0.02	0.02	0.03
		KW=2.30 p=0.51; N.S.						F(36.3)=1.41 p=0.26; N.S.			

N.S.: Not Significant.

Table 8

<table>
<tr><td colspan="12" align="center">ORGAN WEIGHT/BRAIN WEIGHT RATIO DATA SUMMARY</td></tr>
<tr><td colspan="6" align="center">MALE</td><td colspan="6" align="center">FEMALE</td></tr>
<tr><td></td><td></td><td>Group 1</td><td>Group 2</td><td>Group 3</td><td>Group 4</td><td></td><td></td><td>Group 1</td><td>Group 2</td><td>Group 3</td><td>Group 4</td></tr>
<tr><td></td><td></td><td>(0 mg/Kg/day)</td><td>(50 mg/Kg/day)</td><td>(100 mg/Kg/day)</td><td>(200 mg/Kg/day)</td><td></td><td></td><td>(0 mg/Kg/day)</td><td>(50 mg/Kg/day)</td><td>(100 mg/Kg/day)</td><td>(200 mg/Kg/day)</td></tr>
<tr><td></td><td></td><td>N=10</td><td>N=10</td><td>N=10</td><td>N=10</td><td></td><td></td><td>N=10</td><td>N=10</td><td>N=10</td><td>N=10</td></tr>
<tr><td>LIVER
(%)</td><td>MEAN
ST. DEV.</td><td>789.8
137.3</td><td>715.2
101.7</td><td>731.6
103.8</td><td>755.4
121.6</td><td>LIVER
(%)</td><td>MEAN
ST. DEV.</td><td>360.2
33.6</td><td>389.8
39.6</td><td>405.0
41.6</td><td>411.7
54.8</td></tr>
<tr><td></td><td colspan="5" align="center">F(36.3)=0.77 p=0.52; N.S.</td><td></td><td colspan="5" align="center">F(36.3)=2.60 p=0.07; N.S.</td></tr>
<tr><td>HEART
(%)</td><td>MEAN
ST. DEV.</td><td>74.1
11.3</td><td>73.6
10.0</td><td>74.2
15.6</td><td>68.3
8.1</td><td>HEART
(%)</td><td>MEAN
ST. DEV.</td><td>48.2
8.8</td><td>58.0
11.6</td><td>52.5
6.8</td><td>52.9
5.1</td></tr>
<tr><td></td><td colspan="5" align="center">F(36.3)=0.62 p=0.61; N.S.</td><td></td><td colspan="5" align="center">F(36.3)=2.13 p=0.12; N.S.</td></tr>
<tr><td>SPLEEN
(%)</td><td>MEAN
ST. DEV.</td><td>36.8
8.1</td><td>38.6
6.2</td><td>36.4
6.2</td><td>40.7
8.6</td><td>SPLEEN
(%)</td><td>MEAN
ST. DEV.</td><td>29.3
2.9</td><td>32.3
6.6</td><td>30.2
5.4</td><td>29.4
6.1</td></tr>
<tr><td></td><td colspan="5" align="center">F(36.3)=0.71 p=0.55; N.S.</td><td></td><td colspan="5" align="center">F(36.3)=0.62 p=0.61; N.S.</td></tr>
<tr><td>KIDNEYS
(%)</td><td>MEAN
ST. DEV.</td><td>167.6
26.8</td><td>164.1
28.9</td><td>159.5
20.1</td><td>161.6
17.5</td><td>KIDNEYS
(%)</td><td>MEAN
ST. DEV.</td><td>109.3
13.7</td><td>123.4
35.9</td><td>110.6
7.1</td><td>128.5
18.2</td></tr>
<tr><td></td><td colspan="5" align="center">F(36.3)=0.21 p=0.89; N.S.</td><td></td><td colspan="5" align="center">F(36.3)=1.85 p=0.16; N.S.</td></tr>
<tr><td>THYMUS
(%)</td><td>MEAN
ST. DEV.</td><td>41.9
11.5</td><td>33.5
9.0</td><td>39.2
8.8</td><td>37.0
12.6</td><td>THYMUS
(%)</td><td>MEAN
ST. DEV.</td><td>31.4
11.3</td><td>32.6
9.9</td><td>36.6
10.1</td><td>33.9
9.5</td></tr>
<tr><td></td><td colspan="5" align="center">F(36.3)=1.13 p=0.35; N.S.</td><td></td><td colspan="5" align="center">F(36.3)=0.48 p=0.70; N.S.</td></tr>
<tr><td>TESTES
(%)</td><td>MEAN
ST. DEV.</td><td>194.1
28.8</td><td>199.5
24.5</td><td>195.9
20.2</td><td>207.2
29.4</td><td>UTE./CERV.
(%)</td><td>MEAN
ST. DEV.</td><td>56.3
17.5</td><td>59.8
14.3</td><td>46.3
12.6</td><td>45.6
10.1</td></tr>
<tr><td></td><td colspan="5" align="center">F(36.3)=0.48 p=0.70; N.S.</td><td></td><td colspan="5" align="center">F(36.3)=2.63 p=0.06; N.S.</td></tr>
<tr><td>EPIDIDIMS
(%)</td><td>MEAN
ST. DEV.</td><td>132.0
31.1</td><td>117.4
40.5</td><td>108.5
34.1</td><td>130.6
25.0</td><td>OVARIES
(%)</td><td>MEAN
ST. DEV.</td><td>30.6
2.4</td><td>39.9*
8.9</td><td>33.7
6.7</td><td>32.7
6.1</td></tr>
<tr><td></td><td colspan="5" align="center">F(36.3)=1.14 p=0.34; N.S.</td><td></td><td colspan="5" align="center">F(36.3)=3.62 *p<0.05</td></tr>
<tr><td>ADRENALS
(%)</td><td>MEAN
ST. DEV.</td><td>10.9
5.7</td><td>8.2
2.9</td><td>7.4
4.0</td><td>8.7
3.3</td><td>ADRENALS
(%)</td><td>MEAN
ST. DEV.</td><td>11.5
3.6</td><td>12.5
3.3</td><td>13.6
4.0</td><td>13.2
4.9</td></tr>
<tr><td></td><td colspan="5" align="center">F(36.3)=1.35 p=0.27; N.S.</td><td></td><td colspan="5" align="center">F(36.3)=0.54 p=0.66; N.S.</td></tr>
</table>

N.S.: No Significant.