

# Apigenin Ameliorates Lead Acetate induced Hyperlipidemia, Hypothyroidism and Hypogonadism in Male Rats

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## Research Article

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## Abstract

Lead (Pb) is an environmental pollutant and has detrimental effects on human health. Apigenin (APG) is a flavonoid that have antioxidant, anti-inflammatory, antiallergic, and cardioprotective effects and is presented as treatment of some diseases. The aim of the present study was to evaluate the probable protective effect of APG against Pb-induced toxicity in rats. Adult male rats were given either Pb (as lead acetate; 20 mg/kg) alone or in combination with APG (20 mg/kg) daily for 4 weeks by intraperitoneally injection (i.p). At the end of the experimental period, Pb accumulation, lipid profile, thyroid, and testicular function alterations were assessed. In addition, histopathological changes in the testis were assessed. Results revealed that Pb treatment significantly increased Pb concentrations in blood, thyroid gland, and in testis of rats. Further, the blood levels of hormones related to thyroid gland and testis were altered in Pb-treated rats. In parallel, low sperm count and sperm motility, increased sperm abnormalities, and marked pathological changes in testis were observed. On the contrary, the treatment with both Pb and APG recorded amelioration of the deleterious effects of Pb, involving attenuation of changes in lipid profile, thyroidal and testicular hormonal levels, sperm parameters and pathological changes in Pb treated rat's testis. In conclusion, it appears that dietary APG can ameliorate lead acetate induced hyperlipidemia, hypothyroidism and hypogonadism in male rats.

## Introduction

Hyperlipidemia occurred when serum cholesterol or triglycerides levels are elevated and reach levels linked with an increased risk of is chemic heart disease (Walker and Edwards, 2004). Several studies suggested that exposure to lead (Pb) may induce hypercholesterolemia, hypertriglyceridemia and hyperphosphatemia (Vgbaja et al., 2013).

Thyroid gland is an endocrine gland that regulates many body functions by secreting triiodothyronine (T3) and thyroxine (T4) and calcitonin hormones (Tortora and Derrickson, 2017). Previous studies have found that lead has the ability to induce hypothyroidism (Krieg, 2019).

Infertility is a health problem that affects about 15% of couples of reproductive ages (Lukac et al., 2009). Pb is a heavy metal with many industrial uses such as manufacturer of lead acid batteries, coloring agents, paints, smelters, and printing presses (Zhang et al., 2020). It causes environmental pollution and health problems (Okerefor et al., 2020). Pb is a major factor affecting male fertility (Al-Chalabi et al., 2014, El-Nekeety et al., 2009).

Attention has been given to phytotherapy researches to use medical plants with antioxidant activity for protection against heavy metal toxicity (El-Nekeety et al., 2009).

Apigenin (APG) is a natural plant belonging to flavonoids and is found mainly in many plants such as fruits, vegetables and herbs (chamomile celery, celeriac and parsley) (Kabera et al., 2014, Ghitu et al., 2019). It has been documented that Apigenin has antioxidant, anti-inflammatory, anti-lepidemic and anti-apoptic effects (Lee et al., 2007, Shukla et al., 2014, Venigalla et al., 2015, Zhang et al., 2017).

Therefore, the present study was carried out to investigate the protective role of APG against the alteration in lipid profile and toxicity of Pb in testis and thyroid gland of treated rats. This was done using biochemical and structural assessments.

## Materials And Methods

### Chemicals

Lead acetate was purchased from Sigma Chemicals (St. Louis, MO, USA). APG (Cat. No. 520-36-5) was purchased from (Matrix Scientific, Columbia, SC). Levels of total cholesterol (TC), triglycerides (TGs), and high-density lipoprotein cholesterol (HDL-C) were determined by standard kits (BIOMED Diagnostics, Germany). the units were expressed as mg/dL. Malondialdehyde level, as an indicator for lipid peroxidation, Superoxide dismutase, Catalase and glutathione peroxidase activities were determined using commercial kits (catalog # MD2528, SD2520, CA2516, GP2524 respectively) obtained from Diagnostic, Giza, Egypt. TNF  $\alpha$  was determined using Human ELISA Kit (catalog # EA100365) OriGene Technologies Inc., Rockville, MD, IL-6 was determined using Rat Interleukin 6 (IL-6) ELISA Kit (catalog # MBS726707), IL-4 was determined using Rat Interleukin 4 (IL-4) ELISA Kit (catalog # MBS162452), Interleukin 10 (IL-10) was determined using Interleukin 10 (IL-10) ELISA Kit (catalog # MBS764911) MyBioSource, Inc., San Diego, USA. Determination of caspase-3 was done using Biotechne kit (catalog # BF3100) (USA). T4 ELISA kit (Cat. No. 60863) was purchased from Kamiya Biomedical Company, WA, USA. T3 solid-phase competitive ELISA kit (Cat. No. T3043T-100) was obtained from CalbioTech Inc., Spring Valley, Canada. TSH ELISA kit (Cat. No. CSBE05115r) was obtained from CUSABIO Biotech Co., LTD, Wuhan, China. Kit for determination of T was purchased from K-assay, WA, USA. LH and FSH kits were obtained from Biovender, Tokyo, Japan. Determination of caspase-3 was done using Biotechne kit (catalog # BF3100) (USA).

## Animals

Twenty-four adult male rats (*Rattus norvegicus*) weighing 180-200gm were purchased from the animal house of Faculty of Science King Faisal University, Saudi Arabia. All experimental procedures were done according to the research ethics at King Faisal University. The rats were housed in plastic cages (6 per cage), floored with soft wood shaving that was changed three times per week. The animals were acclimatized for 2 weeks prior to the study and were maintained under a 12 h light/dark cycle at (25 °C ± 2 °C), with free access to water and rat chow.

## Preparation of treatment materials:

- Lead acetate dose: dosage of 20mg/kg body weight (b.w.) was dissolved in normal saline and administered intraperitoneally (i.p.) (El-Newehy and El-Sayed, 2011).

- Apigenin dose: Apigenin (20mg/kg b.w.) was dissolved in normal saline and administered (i.p.) (Venigalla et al., 2015, Yang et al., 2017).

## Experimental design:

Animals were randomly divided into four groups of six animals each as follows:

Group I: served as the control group. Rats were daily injected (i.p.) with normal saline (0.9% NaCl) as vehicle.

Group II: Served as lead acetate – treated group. Rats were daily injected (i.p.) with lead acetate dissolved in normal saline at a dose of 20mg/kg b.w.

Group III: Served as lead acetate and Apigenin-treated group. Rats were daily injected (i.p.) with lead acetate dissolved in normal saline at a dose of 20mg/kg (b.w.) followed by injection (i.p.) with Apigenin dissolved in normal saline at a dose of 20mg/kg b.w.

Group IV: Served as Apigenin-treated group. Rats were daily injected (i.p.) with Apigenin dissolved in normal saline at a dose of 20mg/kg b.w.

At the end of the 4-week experimental period, the animals were sacrificed and samples of blood and testis tissues were collected for analysis.

## Estimation of studied parameters:

### a) Evaluation of lead level:

The lead concentration was determined in blood and cerebellum tissue using Atomic Absorption Spectroscopy.

### b) Biochemical parameters:

## I-Determination of serum lipid profile parameters

Levels of total cholesterol (TC), triglycerides (TGs), and high-density lipoprotein cholesterol (HDL-C) were determined by standard kits according to the instructions of the supplier. The levels of LDL-cholesterol (LDL-C) were calculated using Friedewald's formula, the units were expressed as mg/dL.

## Preparation of tissue homogenate

Chosen tissues were immediately removed and washed using chilled saline solution. These tissues were minced and separately homogenized (10 % w/v) in ice-cold sodium-potassium phosphate buffer (0.01 M, pH 7.4) containing 1.15 % KCl using a homogenizer (Potter–Elvehjem). The homogenate was centrifuged at 10,000 xg for 20 min at 4°C and the resultant supernatant was used for the assay of the enzyme activities, the level of MDA and the protein. The protein content of the tissues was determined by the method of Lowry et al. (1951).

## II-Oxidative stress markers

- Activities of catalase, superoxide dismutase, glutathione peroxidase were determined in chosen tissues using commercial kits. The enzymes activities were expressed as U/mg protein. The level of MDA, as an index of lipid peroxidation, was determined through its reaction with thiobarbituric acid (TBA) using the lipid peroxidate kit according to the instruction of the supplier. TBARS values were calculated as nmol MDA per g tissue.

### III- Inflammatory markers

The sera TNF- $\mu$ , IL-6, IL-4 and IL-10 levels were measured using standard kits according to the manufacturer's protocol.

### IV- Apoptosis marker

The levels of caspase-3 enzyme in cerebellum tissue were measured according to the manufacturer's protocol.

### V-Estimation of serum hormonal concentrations

Thyroid hormones were determined by measuring the serum levels of thyroxine (T4), triiodothyronine (T3) and thyroid-stimulating hormone (TSH). T4 was determined following the protocol described in T4 ELISA kit (Cat. No. 60863), Kamiya Biomedical Company, WA, USA. The data of T4 were measured as ng/ml. T3 was estimated using a solid-phase competitive ELISA kit (Cat. No. T3043T-100) obtained from CalbioTech Inc., Spring Valley, Canada. The T3 values were expressed as pg/dl. TSH was estimated using ELISA kit (Cat. No. CSBE05115r) obtained from CUSABIO Biotech Co., LTD, Wuhan, China. The records of TSH were given in  $\mu$ U/ml. Serum levels of testosterone, LH, and FSH were detected according to Sakuma (2009), Shioya and Wakabayashi (1998), and Teerds et al. (1989), respectively. The values of testosterone were expressed as pg/ml whereas LH and FSH values were expressed as ng/ml.

### VI-Assessment of sperm concentration, motility, and abnormality

The left testis of each rat was harvested, then minced in pre-warmed saline (37 °C), and the resulted suspension was used in semen analysis. To analyze sperm motility, 1 drop of sperm suspension was placed on a glass slide to analyze 200 motile sperm in 4 different fields. The motility of the sperm was evaluated microscopically within 2– 4 min of their isolation from the testis, and data were expressed as percentage motility (Morrissey et al. 1988). The sperms were counted using a hemocytometer following the method of Freud and Carol (1964). The technique of Evans and Maxwell (1987) was adopted for sperm abnormality study. Briefly, smears were made by placing a drop from the sperm suspension and one or two drops of the previously warmed (37 °C) eosin–nigrosin stain. The smears were allowed to dry in the air and then examined under the microscope using a high power (100 $\times$ ) oil immersion objective. The normal sperm cells were counted and the percentage was calculated.

### C. Microscopic examination

For preparation of samples used in the examination by light microscope, specimens of testis were collected from all experimental groups and fixed in 10% neutral buffered formalin and routinely processed for stained with hematoxylin and eosin stain (H& E) (Bancroft and Camble, 2002), then sections were examined using light microscope.

### D- Statistical analysis

All variables were compared using one-way analysis of variance (ANOVA) followed by LSD multiple range test. Differences at  $P < 0.05$  were considered as statically significant.

## Results

### Effect of Apigenin on lead levels in the testis and thyroid gland of rats treated with lead acetate

In this study, there was a significant increase in lead level in the serum, thyroid gland and testis tissues of rats treated with lead acetate (GII) relative to control-group (GI). Treatment with APG caused decrease in lead level in serum and the tissues of rats intoxicated with lead acetate

(Table 1). The order of bioaccumulation of lead in selected organs was as follow: Serum> thyroid gland> testis.

## **Effect of Apigenin on lipid profile in sera of rats treated with lead acetate**

Effect of APG on lipid profile in rats treated with lead acetate is shown in Table 2. It is obvious that exposure of rats to lead acetate produced significant increase in the values of TC, TGs and LDL-C, relative to the control group. Also, the results showed that HDL-C level was significantly decreased relative to that obtained for control group. On the contrary, APG treatment attenuated these changes in lipid profile.

## **Effect of APG on oxidative stress markers in testis and thyroid gland tissues of rats treated with lead acetate**

In the present study, as shown in Table 3, rats treated with lead acetate (GII) showed significant decrease in studied antioxidant enzymes activities in thyroid gland and testis tissues as compared to the control group (GI). Also, there was a significant increase in the MDA levels in these tissues as compared to the control group (GI). These changes were markedly attenuated by the treatment with APG.

## **Effect of APG on inflammation response in testis and thyroid gland tissues of rats treated with lead acetate**

Exposure of rats to lead acetate caused increase in levels of proinflammatory cytokines (IL-6 and TNF- $\alpha$ ) and decrease in levels of anti-inflammatory cytokines (IL-4 and IL-10) response in testis and thyroid gland tissues compared to the control group. On the other hand, rats treated with APG showed a significant decrease in the levels of pro-inflammatory cytokines and a significant increase in the levels of anti-inflammatory cytokines in comparison to the lead acetate treated group (Table 4).

## **Effect of Apeginin on caspase 3 in testis and thyroid gland tissues of rats treated with lead acetate**

In this study, there was a significant increase in caspase-3 activities (Table 5) in testis and thyroid gland tissues of rats treated with lead acetate (GII) relative to control- group (GI). These changes were markedly ameliorated by the treatment with APG.

## **Effect of Apigenin on testicular and thyroid gland hormones in sera of rats treated with lead acetate**

In the present study, it was observed a decrease in T, LH and FSH in sera of rats treated with Pb. Furthermore, there was a reduction in levels of T3 and T4 and an increase in TSH level. on the contrary, APG increased the levels of altered hormones in the sera of rats treated with Pb (Table 6).

## **Effect of APG on sperm parameters in rats intoxicated with lead acetate**

The result of this study showed that Lead treatment induced a significant decrease in sperm count and sperm motility and increase in sperm abnormalities. However, the alterations in these sperm parameters were ameliorated in animals that received Pb plus APG when compared to rats treated with Pb alone (Table 7).

## **Control group and Apigenin treated –group:**

In paraffin cross sections of Control & Apigenin rat's testes fixed in formaldehyde and stained with H&E, it was noticed well preserved seminiferous tubules separated by the interstitial tissue (Fig.1,7). Leydig cells occupied the interstitial tissue and each seminiferous tubule was lined with multiple layer of spermatogenic epithelium. The lumina of seminiferous tubules were occupied by the spermatozoa (Fig. 2,8).

## **Lead acetate-treated group:**

In this group, light microscopically examination of H&E testicular sections revealed extremely severe and widespread degenerative changes appeared as deformities of seminiferous tubules lined with wavy outline and surrounded by irregular basement membrane. Also, distinctive appearance of genotoxicity with degenerated interstitial tissue led to large spaces between the seminiferous tubules (Fig.3). Tubules exhibit focal or diffuse extensive necrosis with a few viable cells. The cells were identified in some tubules mostly composed of immature form of germ cells which appeared as small acidophilic cells have darkly stained nuclei. The irregular basement membranes of seminiferous tubules were lined with one or two layers of cells (Fig.4).

## **Lead acetate and Apigenin treated group:**

In contrast, prior Apigenin treatment reduced the degree and the number of necrotic seminiferous tubules. There were relatively well preserved interstitium between seminiferous tubules. The interstitial spaces were narrow and most of the seminiferous tubules regained their normal appearance with more spermatozoa (Figs.6,7). Less improvement was recorded in few seminiferous tubules with depletion of germ cells but with healthy Leydig and spermatogenic cells (Fig.8).

Light micrographs of testis sections fixed in formaldehyde & stained with H&E. (1): control –section showing typical architecture with oval seminiferous tubules (ST) separated by narrow interstitial tissue (arrows) (2) Enlarged part from figure (1) (dashed-circle) showing smooth seminiferous tubules with aggregation of matured spermatozoa in the lumen(S) & sequence of spermatogenic cells. Note, Leydig cells(arrows)(3) lead acetate–section showing distorted arrangement of seminiferous tubules (ST) with wide lytic interstitial tissue (star) .(4) Wavy outline seminiferous tubules showed degeneration of spermatogenic cells with deeply stained nuclei(circle) and deformed spermatozoa (S) .(5) Seminiferous tubules of Lead+ Apigenin –group showing variable degree of healthy units (ST) and restoration of spermatogenesis are evident .Note, well preserved interstitial tissue (arrows) .(6) Enlarged part from figure(5)(dashed-circle) showing oval unite with histopathological pattern of damage in few of seminiferous tubules . Note, healthy Leydig cells (arrows) (7) Seminiferous tubules of Apigenin –group showing nearly normal structure (ST) with an organized distribution of various spermatogenic stages and interstitial tissue. (8) Seminiferous tubules showing germinal epithelium (circle) more or less normal with aggregation of spermatozoa in the lumen (S).

## **Discussion**

### **Effect of Apigenin on lead levels in the testis and thyroid gland**

The present study showed that Pb can be bioaccumulated in the testis and thyroid gland of treated rats as indicated by increased level of Pb in these tissues. Similar results were previously reported by Basalamah et al. (2018). On the other hand, APG treatment can decrease levels of Pb in testis and thyroid gland. This may be due to the ability of APG to chelate Pb (Riceevans et al., 2017).

### **Effect of Apigenin on lipid profile in rat treated with lead acetate**

With respect to the serum lipid profile, results obtained from the present study indicated that lead exposure was associated with increase in the level of total cholesterol, triglycerides and LDL cholesterol and decrease in HDL cholesterol. These results concurred with the findings of (Ige et al., 2019).

It is well known that low level of HDL and high levels of TC, TG and LDL increase the risk of atherosclerosis and cardiovascular diseases (Adeniyi and Soladoye, 2017). Increased level of lipid profile may be due to increased uptake of exogenous cholesterol and subsequent deposition and decreased cholesterol catabolism. The ability of Pb to develop hypercholesterolemia may involve the activation of cholesterol biosynthetic enzymes and simultaneous suppression of cholesterol catabolic enzymes (Ademuyiwa et al., 2005).

On the other hand, APG treatment minimized these changes in lipid profile induced by Pb. This finding is in agreement with that of (El-Barky et al., 2019) in diabetic rats and (Khudiar et al., 2017) in cadmium chloride treated rats. This may be due to the ability of APG to reduce the level of blood fat by promoting cholesterol absorption and conversion, and accelerating reverse cholesterol transport (Zhang et al., 2016).

### **Effect of APG on oxidative stress markers in testis and thyroid gland of rats treated with lead acetate**

The data of the present study showed that Pb can induce oxidative stress in tissues of testes and thyroid gland of treated rats as shown by the observed decrease in the activities of studied antioxidant enzymes and increase in level of MDA in these tissues. This result is in agreement with that reported by Ezejiofor and Orisakwe, (2019) .On the contrary, APG reduced this observed oxidative stress in testis and

thyroid gland. These results agree with the results of Dang et al. (2017) in case of testis of acrylamide treated rats and Panda and Kar (2007) in the thyroid gland of diabetic mice.

The ability of APG treatment to reduce the oxidative stress induced by Pb in testis and thyroid glands may be due to the antioxidant activity of APG which is mainly determined by three hydroxyl groups at its 4', 5, 7 position and double bond at C2 and C3 (Pan et al., 2020).

## **Effect of APG on inflammation response in testis and thyroid gland of rats treated with lead acetate**

The result of the present study showed that Pb treatment caused increase in proinflammatory cytokines levels (TNF- $\alpha$  and IL-6) and decrease in anti-inflammatory cytokines levels (IL-4 and IL-10) in both testis and thyroid gland. These results can be supported by previous finding of Basalamah et al. (2018).

## **Effect of Apeginin on caspase 3 in testis and thyroid gland of rats treated with lead acetate**

Caspases are a family of genes that play a role in maintaining homeostasis by regulating cell death and inflammation (McIlvain et al., 2013).

Our findings clearly showed that the activity of caspase-3 was increases in tests and thyroid gland of rats treated with lead acetate which may indicate that Pb had the ability to induce apoptosis in these tissues. These results are in agreement with Massanyi et al. (2020) studies on Pb apoptic effect in the spermatogenetic cells

The co-administration of Pb and APG decrease the observed apoptic effect of Pb. In parallel with this result, Skondras et al. (2015) demonstrated the ability of APG to attenuate the apoptic effect induced in rat's testis in experimental ischemia-reperfusion. Also, Abdel Kareem and Mahmoud (2017) demonstrated this effect in thyroid of rats intoxicate with deltamethrin.

## **Effect of Apigenin on testicular and thyroid gland hormones in rats treated with lead acetate**

In the present study, a decrease in T, LH and FSH in sera of rats treated with Pb was observed. These findings were supported by the results obtained by Kelainy et al. (2019). Furthermore, there was a a reduction in levels of T3 and T4 and an increase in TSH level. Similar results were obtained by Al-Maathidy et al. (2019).

The decrease in T level by Pb may be due to its ability to produce reactive oxygen species which promote Leydig cells aging and apoptosis. It is well known that Leydig cells are responsible for T secretion (Adekola (2015). The observed alterations in testicular and thyroid gland hormones indicated impairment in function of testis and hypothyroidism.

The observed hypogonadism may be a consequence of hypothyroidism where it is well known that hypothyroidism is associated with a decrease in male sex hormones, abnormal spermatogenesis, and retardation of Sertoli cell differentiation (Mohamed and Gawad, 2017). T3 hormone has the ability to stimulate the basal testosterone biosynthesis by increasing LH receptors and steroidogenesis of Leydig cells (Maran et al. 2000). T3 also regulates mitochondrial function in several tissues (Chattopadhyay et al. 2010).

The observed thyroid dysfunction may be related to structural damage of thyroid follicular cells due to accumulation of Pb in the thyroid gland (Baoliei et al., 2009). The observed increase in serum TSH level is likely a response to decreases serum T4 and T3 level.

On the contrary, APG increased the levels of altered hormones in the sera of rats treated with Pb. This effect can be supported by the finding of Akilah et al. (2018) in testes of rats treated with chloroquinone and Panda and Karin in thyroid gland of diabetic mice.

## **Effect of APG on sperm parameters in rats intoxicated with lead acetate**

In the present study, exposure of rats to Pb showed altered sperm parameter where there were a decrease in sperm count and motility and an increase in sperm abnormality. This result can be supported by that of Oyeyemi et al. (2019).

It is well known that sperm plasma membranes are rich in polyunsaturated fatty acids, containing double bonds vulnerable to free radical attack and initiation of lipid peroxidation which causes impaired membrane structure and functions (Tvrdá et al., 2011, Asadpour et al., 2013).

The observed decrease in sperm count may be due to the ability of Pb to cause oxidative stress and consequently increased death and decreased number of sperms (Tvrda et al., 2011).

Pb may interact with sulfhydryl groups on the proteins of the outer dense fibers and fibrous sheath, which are cytoskeletal components of the flagellum, causing its detachment from the plasma membranes and this may affect the sperm motility (Gomes et al., 2015).

On the other hand, APG treatment ameliorated the alteration in sperm parameters. This may be due to the ability of APG to reduce oxidative stress as demonstrated in this study. Similar result was obtained by (Akilah et al., 2018) in testes of rats treated with chloroquinone.

## **Effect of Apigenin on testis histology**

In the present study, lead acetate led to degenerative changes in the testis of treated rats. This result can be confirmed by those of Soliman et al. (2017) and Ali et al. (2018).

## **Conclusion**

In summary, it can be concluded that 20mg/kg b.w of lead acetate for 4 weeks can induce toxicity in testis and thyroid gland of rats. On the contrary, treatment with APG had (20mg/kg b.w) can attenuate change in lipid profile and had beneficial effect for prophylaxis of Pb-induced testicular and thyroid gland damages.

Results of the current study shows that APG treatment may exhibit these prophylactic effects through anti-hyperlipidemic, antioxidant, anti-inflammatory and anti-apoptic activities of APG.

## **Declarations**

## **Ethical approval**

The experimental protocol of this investigation was approved by Institutional Animal Care and Use Committee (IACUC) at the King Faisal University.

## **Funding**

No funding agency support this research.

## **Declaration of Competing Interest**

The authors report no declarations of interest.

## **Data and materials Availability**

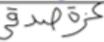
We have no conflict to publish our data

## **Author Contributions**

- Abdulmohsen I. Algefare conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Azza Sedky conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Manal Alfwaaires analyzed the data, contributed reagents/materials/analysis tools, authored or reviewed drafts of the paper, approved the final draft.
- Omar Mahmoud analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.

## Consent to Participate

Consent to Participate I/we certify that I/we have participated sufficiently in the intellectual content, conception and design of this work or the analysis and interpretation of the data (when applicable), as well as the writing of the manuscript, to take public responsibility for it and have agreed to have my/our name listed as a contributor. I/we believe the manuscript represents valid work. Neither this manuscript nor one with substantially similar content under my/our authorship has been published or is being considered for publication elsewhere, except as described in the covering letter. I/we certify that all the data collected during the study is presented in this manuscript and no data from the study has been or will be published separately. I/we attest that, if requested by the editors, I/we will provide the data/information or will cooperate fully in obtaining and providing the data/information on which the manuscript is based, for examination by the editors or their assignees. Financial interests, direct or indirect, that exist or may be perceived to exist for individual contributors in connection with the content of this paper have been disclosed in the cover letter. Sources of outside support of the project are named in the cover letter. I/We hereby transfer(s), assign(s), or otherwise convey(s) all copyright ownership, including any and all rights incidental thereto, exclusively to the Journal, in the event that such work is published by the Journal. The Journal shall own the work, including 1) copyright; 2) the right to grant permission to republish the article in whole or in part, with or without fee; 3) the right to produce preprints or reprints and translate into languages other than English for sale or free distribution; and 4) the right to republish the work in a collection of articles in any other mechanical or electronic format. We give the rights to the corresponding author to make necessary changes as per the request of the journal, do the rest of the correspondence on our behalf and he/she will act as the guarantor for the manuscript on our behalf. All persons who have made substantial contributions to the work reported in the manuscript, but who are not contributors, are named in the Acknowledgment and have given me/us their written permission to be named. If I/we do not include an Acknowledgment that means I/we have not received substantial contributions from non-contributors and no contributor has been omitted

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## Consent to Publish

The authors report no conflict to publish

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## Tables

**Table (1) The effect of apigenin on the lead level in sera, testis and thyroid gland of rats intoxicated with lead acetate**

		Serum ( $\mu\text{g/ml}$ )	Testis ( $\mu\text{g/g tissue}$ )	Thyroid gland ( $\mu\text{g/g tissue}$ )
<b>Group I</b>	<b>6</b>	0.14 <sup>c</sup> $\pm$ 0.01	0.13 <sup>c</sup> $\pm$ 0.01	0.16 <sup>c</sup> $\pm$ 0.01
<b>Group II</b>	<b>6</b>	5.4 <sup>a</sup> $\pm$ 0.08	3.4 <sup>a</sup> $\pm$ 0.07	4.3 <sup>a</sup> $\pm$ 0.08
<b>Group III</b>	<b>6</b>	1.5 <sup>b</sup> $\pm$ 0.06	0.96 <sup>b</sup> $\pm$ 0.03	1.2 <sup>b</sup> $\pm$ 0.05
<b>Group IV</b>	<b>6</b>	0.12 <sup>c</sup> $\pm$ 0.01	0.13 <sup>c</sup> $\pm$ 0.01	0.14 <sup>c</sup> $\pm$ 0.01
<b>F (p)</b>		2410.410*(<0.001*)	1787.467*(<0.001*)	1676.828*(<0.001*)

Normally distributed data was expressed in **Mean  $\pm$  SE.**

**F: F for ANOVA test**, Pairwise comparison bet. each 2 groups was done using **Post Hoc Test (Tukey)**

p: p value for comparing between the studied groups

Means with **Common letters** are not significant (Means with **Different letters** are significant)

\*: Statistically significant at  $p \leq 0.05$

**Table (2): Effect of Apigenin on lipid profile in sera of rats treated with lead acetate**

	No.	TC (mg/dL)	TGs (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)
Group I	6	54.3 <sup>c</sup> ± 0.2	36.3 <sup>b</sup> ± 0.2	29.2 <sup>a</sup> ± 0.2	17.1 <sup>c</sup> ± 0.2
Group II	6	114.5 <sup>a</sup> ± 0.2	76.2 <sup>a</sup> ± 0.2	17.4 <sup>c</sup> ± 0.1	56.3 <sup>a</sup> ± 0.1
Group III	6	66.1 <sup>b</sup> ± 0.3	48.8 <sup>c</sup> ± 0.1	20.1 <sup>b</sup> ± 0.1	29.5 <sup>b</sup> ± 0.1
Group IV	6	54.9 <sup>c</sup> ± 0.1	36.5 <sup>b</sup> ± 0.2	28.9 <sup>a</sup> ± 0.1	17.6 <sup>c</sup> ± 0.2
F (p)		18921.525* (<0.001*)	10981.932* (<0.001*)	2185.817* (<0.001*)	17914.596* (<0.001*)

Normally distributed data was expressed in **Mean ± SE**.

**F: F for ANOVA test**, Pairwise comparison bet. each 2 groups was done using **Post Hoc Test (Tukey)**

p: p value for comparing between the studied groups

Means with **Common letters** are not significant (Means with **Different letters** are significant)

\*: Statistically significant at  $p \leq 0.05$

**Table (3): Effect of Apigenin on oxidative stress markers induced in testis and thyroid gland tissues of rats treated with lead acetate**

	No.	Testis				Thyroid gland			
		CAT (U/mg protein)	SOD (U/mg protein)	GPX (U/mg protein)	MDA (nmol/g tissue)	CAT (U/mg protein)	SOD (U/mg protein)	GPX (U/mg protein)	MDA (nmol/g tissue)
Group I	6	45.0 <sup>a</sup> ± 0.45	65.74 <sup>a</sup> ± 0.26	64.70 <sup>a</sup> ± 0.30	12.72 <sup>c</sup> ± 0.4	44.40 <sup>a</sup> ± 0.10	45.22 <sup>a</sup> ± 0.09	65.52 <sup>a</sup> ± 0.29	14.60 <sup>c</sup> ± 0.5
Group II	6	21.0 <sup>c</sup> ± 0.45	27.20 <sup>c</sup> ± 0.37	24.80 <sup>c</sup> ± 0.24	33.70 <sup>a</sup> ± 0.30	15.52 <sup>b</sup> ± 0.14	27.28 <sup>c</sup> ± 0.14	20.24 <sup>c</sup> ± 0.10	35.38 <sup>a</sup> ± 0.12
Group III	6	36.02 <sup>b</sup> ± 0.27	46.20 <sup>b</sup> ± 0.37	54.86 <sup>b</sup> ± 0.21	22.58 <sup>b</sup> ± 0.4	31.60 <sup>c</sup> ± 0.13	37.60 <sup>b</sup> ± 0.51	46.18 <sup>b</sup> ± 0.10	24.38 <sup>b</sup> ± 0.8
Group IV	6	44.80 <sup>a</sup> ± 0.37	66.0 <sup>a</sup> ± 0.32	63.60 <sup>a</sup> ± 0.29	12.02 <sup>c</sup> ± 0.19	42.24 <sup>a</sup> ± 0.10	44.46 <sup>a</sup> ± 0.16	63.38 <sup>a</sup> ± 0.16	14.40 <sup>c</sup> ± 0.12
F (p)		969.096* (<0.001*)	3272.599* (<0.001*)	5754.810* (<0.001*)	5714.827* (<0.001*)	12622.152* (<0.001*)	7397.031* (<0.001*)	14572.984* (<0.001*)	23360.667* (<0.001*)

Normally distributed data was expressed in **Mean ± SE**.

**F: F for ANOVA test**, Pairwise comparison bet. each 2 groups were done using **Post Hoc Test (Tukey)**

p: p value for comparing between the studied groups

Means with **Common letters** are not significant (Means with **Different letters** are significant)

\*: Statistically significant at  $p \leq 0.05$

**Table (4): Effect of Apigenin on inflammatory response in testis and thyroid gland tissues of rats treated with lead acetate**

	No.	Testis				Thyroid gland			
		Pro-Inflammatory		Anti-Inflammatory		Pro-Inflammatory		Anti-Inflammatory	
		IL-6	TNF- $\alpha$	IL-4	IL-10	IL-6	TNF- $\alpha$	IL-4	IL-10
<b>Group I</b>	<b>6</b>	51.3 <sup>c</sup> $\pm$ 0.14	45.30 <sup>c</sup> $\pm$ 0.14	49.30 <sup>a</sup> $\pm$ 0.14	39.32 <sup>a</sup> $\pm$ 0.13	44.20 <sup>b</sup> $\pm$ 0.09	45.40 <sup>c</sup> $\pm$ 0.19	53.02 <sup>a</sup> $\pm$ 0.24	34.50 <sup>a</sup> $\pm$ 0.22
<b>Group II</b>	<b>6</b>	241.2 <sup>a</sup> $\pm$ 0.46	226.8 <sup>a</sup> $\pm$ 0.25	22.36 <sup>c</sup> $\pm$ 0.12	19.32 <sup>c</sup> $\pm$ 0.14	202.7 <sup>a</sup> $\pm$ 0.59	227.6 <sup>a</sup> $\pm$ 0.19	26.0 <sup>c</sup> $\pm$ 0.35	17.60 <sup>c</sup> $\pm$ 0.29
<b>Group III</b>	<b>6</b>	101.14 <sup>b</sup> $\pm$ 0.3	144.20 <sup>b</sup> $\pm$ 0.3	36.74 <sup>b</sup> $\pm$ 0.25	28.40 <sup>b</sup> $\pm$ 0.19	104.6 <sup>c</sup> $\pm$ 0.29	145.6 <sup>b</sup> $\pm$ 0.19	33.30 <sup>b</sup> $\pm$ 0.37	24.64 <sup>b</sup> $\pm$ 0.19
<b>Group IV</b>	<b>6</b>	53.2 <sup>c</sup> $\pm$ 0.34	44.6 <sup>c</sup> $\pm$ 0.50	47.0 <sup>a</sup> $\pm$ 0.18	37.60 <sup>a</sup> $\pm$ 0.29	43.7 <sup>b</sup> $\pm$ 0.14	47.6 <sup>c</sup> $\pm$ 0.20	55.60 <sup>a</sup> $\pm$ 0.58	35.34 <sup>a</sup> $\pm$ 0.15
<b>F (p)</b>		300063.477* ( $<$ 0.001*)	151836.120* ( $<$ 0.001*)	5630.931* ( $<$ 0.001*)	2202.133* ( $<$ 0.001*)	405960.114* ( $<$ 0.001*)	897517.540* ( $<$ 0.001*)	1046.809* ( $<$ 0.001*)	1391.122* ( $<$ 0.001*)

Normally distributed data was expressed in **Mean  $\pm$  SE.**

**F: F for ANOVA test**, Pairwise comparison bet. each 2 groups were done using **Post Hoc Test (Tukey)**

p: p value for comparing between the studied groups

Means with **Common letters** are not significant (Means with **Different letters** are significant)

\*: Statistically significant at  $p \leq 0.05$

The inflammatory cytokines are expressed as pg/mg protein.

**Table (5): Effect of Apigenin on caspase-3 activities in testis and thyroid gland tissues of rats treated with lead acetate**

	No.	Caspase-3 activities (ng/mg protein)	
		Testis	Thyroid gland
		<b>Group I</b>	<b>6</b>
<b>Group II</b>	<b>6</b>	56.90 <sup>a</sup> $\pm$ 0.40	71.98 <sup>a</sup> $\pm$ 0.10
<b>Group III</b>	<b>6</b>	32.04 <sup>b</sup> $\pm$ 0.13	47.70 <sup>b</sup> $\pm$ 0.13
<b>Group IV</b>	<b>6</b>	23.30 <sup>c</sup> $\pm$ 0.09	21.62 <sup>c</sup> $\pm$ 0.12
<b>F (p)</b>		5490.826* ( $<$ 0.001*)	37860.259* ( $<$ 0.001*)

Normally distributed data was expressed in **Mean  $\pm$  SE.**

**F: F for ANOVA test**, Pairwise comparison bet. each 2 groups was done using **Post Hoc Test (Tukey)**

p: p value for comparing between the studied groups

Means with **Common letters** are not significant (Means with **Different letters** are significant)

\*: Statistically significant at  $p \leq 0.05$

**Table (6): Effect of Apigenin on some testicular and thyroid gland hormones in sera of rats treated with lead acetate**

	No.	Testicular hormones			Thyroid gland hormones		
		T	LH	FSH	T3	T4	TSH
		(pg/ml)	(ng/ml)	(ng/ml)	pg/dl	ng/ml	μIU/ml
<b>Group I</b>	<b>6</b>	8.52 <sup>a</sup> ± 0.12	1.50 <sup>a</sup> ± 0.05	0.87 <sup>a</sup> ± 0.01	121.8 <sup>a</sup> ± 0.26	9.42 <sup>a</sup> ± 0.08	7.42 <sup>b</sup> ± 0.07
<b>Group II</b>	<b>6</b>	4.71 <sup>c</sup> ± 0.01	0.69 <sup>c</sup> ± 0.01	0.26 <sup>c</sup> ± 0.01	55.30 <sup>c</sup> ± 0.10	3.37 <sup>d</sup> ± 0.05	10.46 <sup>a</sup> ± 0.07
<b>Group III</b>	<b>6</b>	6.28 <sup>b</sup> ± 0.06	0.9 <sup>b</sup> ± 0.02	0.57 <sup>b</sup> ± 0.01	97.2 <sup>b</sup> ± 0.37	7.12 <sup>a</sup> ± 0.10	5.38 <sup>c</sup> ± 0.04
<b>Group IV</b>	<b>6</b>	8.32 <sup>a</sup> ± 0.09	1.4 <sup>a</sup> ± 0.0	0.89 <sup>a</sup> ± 0.0	124 <sup>a</sup> ± 0.55	9.34 <sup>c</sup> ± 0.05	7.38 <sup>b</sup> ± 0.04
<b>F (p)</b>		2572.732* (<0.001*)	171.819* (<0.001*)	1719.320* (<0.001*)	7528.183* (<0.001*)	1488.571* (<0.001*)	975.458* (<0.001*)

Normally distributed data was expressed in **Mean ± SE**.

**F: F for ANOVA test**, Pairwise comparison bet. each 2 groups was done using **Post Hoc Test (Tukey)**

p: p value for comparing between the studied groups

Means with **Common letters** are not significant (Means with **Different letters** are significant)

\*: Statistically significant at  $p \leq 0.05$

**Table (7): Effect of Apigenin on sperm parameters in rats treated with lead acetate**

	No.	Sperm		
		Count	Motility	Abnormality
		(million cells/ml)	(%)	(%)
<b>Group I</b>	<b>5</b>	95.4 <sup>a</sup> ± 0.8	83.4 <sup>a</sup> ± 0.8	8.0 <sup>c</sup> ± 0.4
<b>Group II</b>	<b>5</b>	62.4 <sup>c</sup> ± 0.7	55.2 <sup>c</sup> ± 1.0	43.2 <sup>a</sup> ± 1.0
<b>Group III</b>	<b>5</b>	75.0 <sup>b</sup> ± 1.0	74.6 <sup>b</sup> ± 0.7	21.8 <sup>b</sup> ± 0.7
<b>Group IV</b>	<b>5</b>	95.2 <sup>a</sup> ± 0.9	85.2 <sup>a</sup> ± 0.8	8.2 <sup>c</sup> ± 0.4
<b>F (p)</b>		332.051* (<0.001*)	269.60* (<0.001*)	603.927* (<0.001*)

Normally distributed data was expressed in **Mean ± SE**.

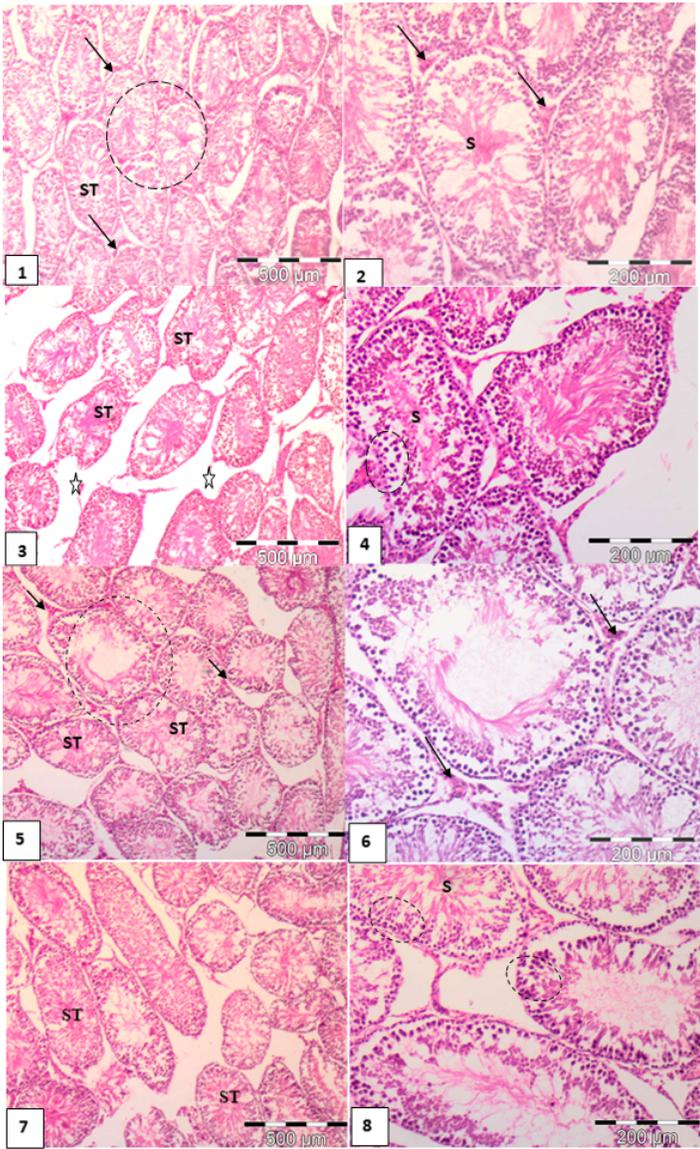
**F: F for ANOVA test**, Pairwise comparison bet. each 2 groups was done using **Post Hoc Test (Tukey)**

p: p value for comparing between the studied groups

Means with **Common letters** are not significant (Means with **Different letters** are significant)

\*: Statistically significant at  $p \leq 0.05$

## Figures



**Figure 1**

Please See the Supplemental Files section for the complete figure caption