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Apigenin Ameliorates Lead Acetate Induced Hyperlipidemia and Hypogonadism in Male Rats

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ABSTRACT

Background: Lead (Pb) is an environmental pollutant and has detrimental effects on human health. Apigenin (APG) is a flavonoid that have antioxidant, anti-inflammatory and antiallergic and cardioprotective so is used as treatment of many diseases. The aim of the present study was to evaluate the probable protective effect of APG against Pb-induced toxicity in rats.

Methods: 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 and testicular function alterations were assessed. In addition, histopathological changes in the testis were assessed.

Result: Results revealed that Pb treatment significantly increased Pb concentrations in blood and testis of rats. Further, the blood levels of hormones related to 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 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 and hypogonadism in male rats.

Key words: Apigenin, Lead, Lipid profile, Testis.

INTRODUCTION

It has been well-acknowledged that heavy metal accumulation in organisms exhibits cytotoxic effects and leads to pathological alterations of organ and cell at both cellular and molecular levels (Chen *et al*., 2020). Lead 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 (Okereafor *et al*., 2020). Pb is a major factor affecting male fertility (Al-Chalabi *et al*., 2014). Infertility is a health problem that affects about 15% of couples of reproductive ages (Lukac *et al.,* 2009).

Several evidence were showing the risk of lead toxicity on male reproductive system. Such as, the lumens devoid of sperm in testicular architecture, conspicuous degenerative changes in the testis, seminiferous epithelium degeneration, reduced number of epithelium spermatozoa, sharp depressions,membrane folding and granularity at sperm head surfaces were perceived (Kumar and Devi, 2018). Also, several studies suggested that exposure to lead (Pb) may induce hypercholesterolemia, hypertriglyceridemia and hyperphosphatemia (Ugbaja *et al*., 2013).

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. It is found mainly in many plants such as fruits, vegetables and herbs (chamomile celery, celeriac and parsley) (Lee *et al*., 2007). It has been documented that Apigenin has antioxidant, anti-inflammatory, anti-lepidemic and anti-apoptic effects (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 of treated rats. This was done using biochemical and structural assessments.

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MATERIALS AND METHODS

Animals

Twenty-four adult male rats (*Rattus norvegicus*) weighing 180-200 gm 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 with reference number: KFU-REC-2021-DEC-EA000332. The rats were housed in plastic cages (6 per cage), floored with soft a wood shaving that was changed three times per week. The animals were acclimatized for 2 weeks prior 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 20 mg/kg body weight (b.w.) was dissolved in normal saline and administrated intraperitoneally (i.p.) (El Neweshy and El Sayed, 2011).

Apigenin dose

Dosage of 20 mg/kg body weight (b.w.) was dissolved in normal saline and administrated intraperitoneally (i.p.) (Yang *et al*., 2017; Zhang *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% Na Cl) 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 20 mg/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 20 mg/kg (b.w.) followed by injection (i.p) with Apigenin dissolved in normal saline at dose of 20 mg/kg b.w.

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

At the end of the 4-week experimental period, the rats were deprived of food for 12 h, then sacrificed and samples of blood and testis tissues were collected for analysis.

Estimation of studied parameters

Evaluation of lead level in serum and testis

The lead concentration was determined in serum and testis by spectrophotometric method as previously reported (Jin *et al*., 2009).

Biochemical parameters

I-Determination of serum lipid profile parameters

The serum concentration of total cholesterol was determined by enzymatic methods. The serum high-density lipoprotein (HDL) content was determined in the supernatant fraction after precipitation of the very low-density lipoproteins (VLDL) and low-density lipoproteins (LDL) with phosphotungstic acid and $MgCl_2$. LDL-cholesterol and VLDL-cholesterol values were calculated according to Friedewald *et al*. (1972).

II-Oxidative stress markers

Activities of catalase, superoxide dismutase, glutathione peroxidase and lipide peroxidation were determined in testis tissues using commercial kits. The protein contents of testis were determined by the method of Lowry *et al*. (1951). Using bovine serum albumin as a standard.

III- Inflammatory markers

The presence of TNF-α IL-4, IL-10 and IL-6 in the cell supernatant was measured with a rat standard-ELISA kit.

IV-Estimation of serum hormonal concentrations

Serum levels of testosterone, LH and FSH were detected according to Sakuma, Shioya and Wakabayashi (1998).

VI-Assessment of sperm concentration, motility and abnormality

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.

Microscopic examination

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 (Hand E) (Bancroft and Gamble, 2002).

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 AND DISCUSSION

Effect of Apigenin on lead levels in the sera and testis of rats treated with lead

In this study, there was a significant increase in lead level in the serum and testis tissues of rats treated with lead. Treatment with APG caused decrease in lead level in serum and the tissues of rats intoxicated with lead This may be due to the ability of APG to chelate Pb (Table 1). The order of bioaccumulation of lead in selected organs was as follow: Serum> testis. Similar results were previously reported by Basalamah *et al.* (2018).

Table 1: The effect of Apigenin on the lead level in sera, testis of rats treated with lead.

	No.	Serum (µg/ml)	Testis (µg/gm)
Control	6	0.14^{c}±0.01	0.13^{c}±0.01
Lead	6	5.43^{a}±0.08	3.41^{a}±0.07
Lead+ Apigenin	6	1.45^{b}±0.06	0.96^{b}±0.03
Apigenin	6	0.12^{c}±0.01	0.13^{c}±0.01
F (p)		2410.410 *(<0.001*)	1787.467* (<0.001*)

Data are presented as means s ± SE. N = six experimental animals per group; Mean values with similar letters are insignificant. *Statistically significant at P≤0.05.

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

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 produced significant increase in the values of TC, TGs and LDL-C and decrease in HDL-C level. These results concurred with the findings of Ige *et al*. (2019).

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). 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.,* 2017).

Effect of APG on oxidative stress markers in testis of rats treated with lead

As shown in Table 3 rats treated with lead showed significant decrease in studied antioxidant enzymes activities and increase in the MDA levels in testis.

Generation of excessive reactive oxygen species due to lead exposure potentially affects spermatozoa viability, motility, DNA fragmentation and chemotaxis for spermatozoa–oocyte fusion, all of which can contribute to deterring fertilization (Gandhi *et al.,* 2017).

The observed changes were markedly attenuated by the treatment with APG. This result is in agreement with that reported by Ezejiofor and Orisakwe (2019). On the contrary, APG reduced this observed oxidative stress in testis. These results agree with the results of Dang *et al.* (2017).

The ability of APG treatment to reduce the oxidative stress induced by Pb in testis 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 of rats treated with lead

Exposure of rats to lead acetate caused increase in levels of proinflammatory cytokines and decrease in levels of anti-inflammatory cytokines. On the other hand, rats treated with APG showed amelioration (significant at P≤0.05) in the levels of these cytokines (Table 4). These results can be supported by previous finding of Basalamah *et al.* (2018).

Effect of Apigenin on some testicular related hormone in sera of rats treated with lead

In the present study, there were an observed decrease in T, LH and FSH in sera of rats treated with Pb. On the contrary, APG increased the levels of altered hormones in the sera of rats treated with Pb (Table 5). These findings were supported by the results obtained by Kelain *et al*. (2019). The observed alterations in testicular hormones indicated impairment in function of testis. 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 signals within and between the

Table 2: Effect of Apigenin on lipid profile in sera of rats treated with lead.

	No.	TC (mg/dl)	TGs (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
Control	6	86.17^{c}±0.54	58.0^{c}±0.52	47.83^{c}±0.48	34.83^{c}±0.60	10.90^{c}±0.34
Lead	6	174.8^{a}±0.87	135.0^{a}±1.73	32.67^{b}±0.95	107.2^{a}±0.70	26.70^{a}±0.51
Lead + Apigenin	6	105.5^{b}±0.43	85.17^{b}±0.70	38.33^{a}±0.49	57.67^{b}±0.49	19.0^{b}±0.45
Apigenin	6	86.17^{c}±0.60	59.83^{c}±0.65	46.50^{c}±0.92	36.17^{c}±0.60	10.58^{c}±0.45
F (p)		4432.963	1229.089*	91.376	3127.471*	299.735
		(<0.001)	(<0.001*)	*(<0.001*)	(<0.001*)	*(<0.001*)

Total cholesterol: TC, TG: triglyceride, serum high-density lipoprotein: HDL-C, very low-density lipoproteins VLDL-C, low-density lipoproteins LD-CL. Data are presented as means s ± SE. N = six experimental animals per group. Mean values with similar letters are insignificant. *Statistically significant at P≤0.05.

Table 3: Effect of Apigenin on oxidative stress markers induced in testis tissue of rats treated with lead.

	No.	Testis			
		CAT (U/mg protein)	SOD (U/mg protein)	GPX (U/mg protein)	MDA (nmol/g tissue)
Group I	6	45.0^{a}±0.45	65.74^{a}±0.26	64.70^{a}±0.30	12.72^{c}±0.4
Group II	6	21.0^{c}±0.45	27.20^{c}±0.37	24.80^{c}±0.24	33.70^{a}±0.30
Group III	6	36.02^{b}±0.27	46.20^{b}±0.37	54.86^{b}±0.21	22.58^{b}±0.4
Group IV	6	44.80^{a}±0.37	66.0^{a}± 0.32	63.60^{a}±0.29	12.02^{c}±0.19
F (p)		969.096*	3272.599*	5754.810*	5714.827
		(<0.001*)	(<0.001*)	(<0.001*)	*(<0.001*)

Catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), Malondialdehyde (MDA); Data are presented as means s ± SE. N = six experimental animals per group; Mean values with similar letters are insignificant. *Statistically significant at P≤0.05.

Table 4: Effect of Apigenin on inflammatory response in testis of rats treated with lead.

	No.	Testis			
		Pro-inflammatory		Anti-inflammatory	
		TNF-α (pg/mg protein)	IL-6 (pg/mg protein)	IL-4 (pg/mg protein)	IL-10 (pg/mg protein)
Control	6	51.0^{c}±0.58	45.67^{c}±0.80	23.0^{c}± 0.58	37.17^{a}±0.65
Lead	6	239.7^{a}±0.88	242.3^{a}±0.61	36.33^{b}±0.31	17.0^{c}±0.68
Lead + API	6	103.5^{b}±1.02	147.2^{b}±0.95	47.33^{a}±0.49	27.50^{b}±0.56
Apigenin	6	51.83^{c}±0.48	46.17^{c}±1.08	23.83^{c}±0.65	37.0^{a}±0.58
F (p)		13234.078* (<0.001*)	11558.051 *(<0.001*)	484.874 *(<0.001*)	237.554 *(<0.001*)

Tumor necrotic factor-alpha: TNF-α, Interleukine-4: IL-4, Interleukine-10: IL-10, Interleukine-6: IL-6; Data are presented as means s ± SE. N = Six experimental animals per group; Mean values with similar letters are insignificant. *Statistically significant at P≤0.05.

hypothalamus and pituitary gland appear to be disturbed by long-term, low-dose Lead exposure (Sokol *et al.,* 2002).

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).

Effect of APG on sperm parameters in rats intoxicated with lead

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, APG ameliorated these alterations (Table 6).

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). Lead treatment inhibited the spermatogenesis by reducing spermiation (VII and VIII) and beginning of mitosis (IX-XI) process length (Leiva *et al*., 2011).

In human studies, it was found that the spermatogenesis impairment may due to an excessive amount of lead exposure on Sertoli cells which might be a result of inhibin B overproduction (Mahmoud *et al.,* 2005). Prolonged exposure of lead to male rats revealed the deterioration of spermatogenesis in addition with Leydig cell degeneration. The germinal function during the growing stages of testis at maturity is altered because of the disturbed steroidogenesis (Saxena *et al.,* 1987).

Effect of Apigenin on testis histology

Control group and Apigenin treated-group

Showed well preserved seminiferous tubules separated by the interstitial tissue. Each seminiferous tubule was lined with multiple layers of spermatogenic epithelium. The lumina of seminiferous tubules were occupied by the spermatozoa (Fig 1a, 1b, 4a, 4b).

Lead-treated group

There were extremely severe and widespread degenerative changes appeared as deformities of seminiferous tubules lined with wavy outline and surrounded by irregular basement membrane. Also, degenerated interstitial tissue is present leading to large spaces between the seminiferous tubules (Fig 2a). Affected tubules were lined with one or two layers of germinal cells (Fig 2b).

Lead and Apigenin treated group

Apigenin treatment reduced the degree of alterations in many seminiferous tubule that exhibited well-preserved structure with preserved spermatogenesis and relatively well-preserved interstitial between seminiferous tubules

Table 5: Effect of Apigenin on some testicular hormones in sera of rats treated with lead.

	No	Testicular hormones		
		·T (pg/ml)	LH (ng/ml)	FSH (ng/ml)
Group I	6	8.52^{a}±0.12	1.50^{a}±0.05	0.87^{a}±0.01
Group II	6	0.71^{c}±0.01	0.69^{c}±0.01	0.26^{c}±0.01
Group III	6	8.28^{a}±0.06	1.46^{a}±0.02	0.87^{a}± 0.01
Group IV	6	2.32^{b}±0.09	0.94^{b}±0.0	0.59^{b}±0.0
F (p)		2572.732 *(<0.001*)	171.819 *(<0.001*)	1719.320 *(<0.001*)

Testosterone: T, Luteinizing hormone: LH, Follicle stimulating hormone: FSH; Data are presented as means s ± SE. N = six experimental animals per group; Mean values with similar letters are insignificant. *Statistically significant at P≤0.05.

Table 6: Effect of Apigenin on sperm parameters in rats treated with lead acetate.

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

Data are presented as means ± SE. N = Six experimental animals per group; Mean values with similar letters are insignificant. *Statistically significant at P≤0.05.

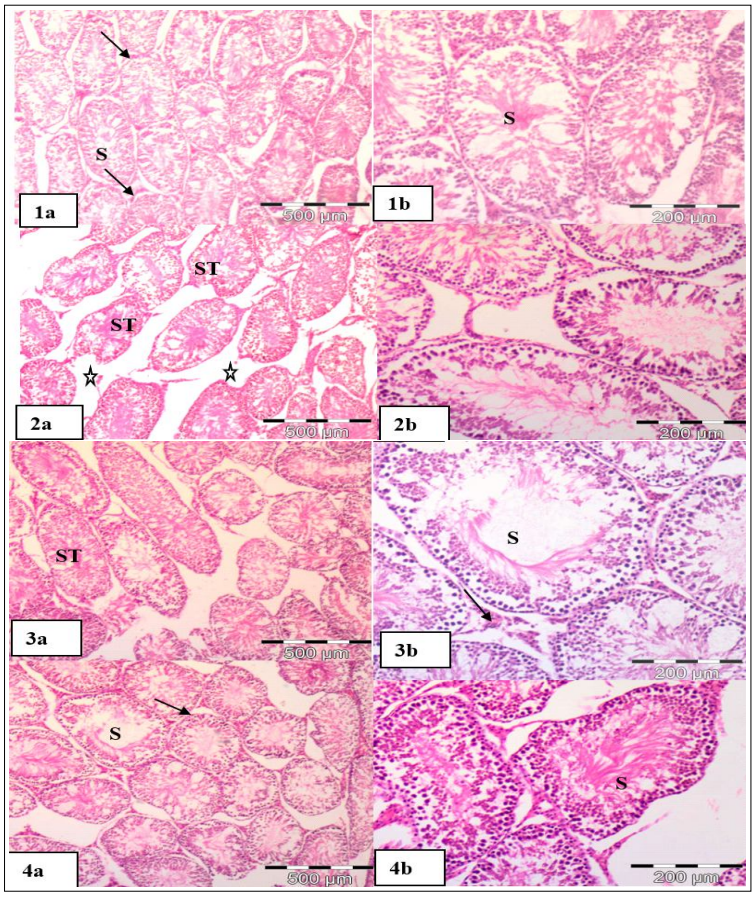

Fig 1-4: Hematoxylin and eosin staining of testicular tissue of different experimental groups. 1a, 1b control, 4a, 4b Apigenin treated group viewing normal structure of seminiferous tubules with complete spermatogenic series and interstitial tissue.2a, 2b lead treated group demonstrating testicular disruption. 3a, 3b lead and Apigenin treated group indicating the improvement in the seminiferous structure. ST, seminiferous tubules, interstitial tissue (arrow), S, lumen, dashed circle, germinal epithelium, astric, lytic interstitial tissue. (500 µm=X40, 200 µm=X100).

(Fig 3a, 3b). This result can be confirmed by those of Ali *et al*. (2018).

CONCLUSION

In summary, it can be concluded that lead induce toxicity in testis of rats. On the contrary, treatment with APG can attenuate change in lipid profile and Pb-induced testicular damages. APG treatment may exhibit these prophylactic effects through anti-hyperlipidemic, antioxidant and anti-inflammatory activities of APG.

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Ethical statement

The experimental protocol of this investigation was approved by Institutional Animal Care and Use Committee (IACUC) at the King Faisal University with Research Ethics Committee number: KFU-REC/2021-03-05.

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Conflict of Interest: None.

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