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Article

Effects of flubendiamide and lead exposure on circulating thyroid hormone levels in buffalo (*Bubalus bubalis*) calves

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Abstract: The current investigation aimed to find how exposure to lead and flubendiamide affected the amounts of thyroid hormone levels in buffalo calves' blood. For ninety days, male buffalo calves were given lead acetate orally at a rate of 9.2 mg/kg bw/day and flubendiamide orally at a rate of 0.024 mg/kg bw/day, either in combination or separately. T3 and T4 levels in blood decreased in response to both flubendiamide and lead exposure alone. When lead and flubendiamide were administered together, the animals' T3 and T4 levels declined less than when lead and flubendiamide were administered separately. TSH activity increased markedly in rats exposed to both lead and flubendiamide, but not consistently in animals treated with flubendiamide alone. Results of the present study indicated thyrotoxic potential of flubendiamide and lead in buffalo calves. However, further study is required to elucidate the mechanism of thyrotoxic potential of flubendiamide and to understand the interactive effects of these two toxicants on thyroid function in mammals.

Keywords: flubendiamide; lead; pesticide; thyroid-hormone; toxicity

1. Introduction

A new insecticide called flubendiamide, a member of the phthalic acid diamide family was released in the international market in July 2005, and in 2007 in India [1]. Three novel substituents contribute to the distinctive chemical structure of flubendiamide: An iodine atom at the 3-position of the phthalic acid moiety, a sulfonyl alkyl group in the aliphatic amide moiety, and a heptafluoro isopropyl group in the anilide moiety [2].

More than a hundred synthetic and naturally occurring chemicals have been shown to affect thyroid hormone metabolism or thyroid function thus far [3]. Numerous studies have shown a negative association between blood lead and thyroid hormone levels in the human population [4,5]. Similarly, fish exposed to Pb have lower amounts of thyroid hormones in their blood [6,7]. Although flubendiamide has been shown to affect haematological indices in Sprague Dawley Rats [8], buffalo calves [9,10], induce oxidative stress [11], and change the pharmacokinetics of cefquinome in buffalo calves [12], there seem no published study examining its effects on the status of circulating thyroid hormone levels in mammals.

The possibility that a combination of endocrine disrupting substances can have major impacts even when they are present at concentrations that do not individually cause any discernible effects is becoming more widely acknowledged [13]. Comparatively little is known about combinations made up of substances from

several classes of endocrine disruptors, as the majority of investigations have focused on the combined effects of endocrine-disruptors that fall under the same group [14]. We postulated that flubendiamide might interact with lead-induced alterations in thyroid hormone levels in the bloodstream.

2. Materials and methods

2.1. Animals

Sixteen healthy male buffalo calves (8 to 12 months old), weighting in between 120 kg–180 kg were used as experimental animals in the present study. Before the trial began in the department's experimental animal house, they were given a balanced diet, dewormed, and given two weeks for acclimatization to the new surroundings. The blood and faecal samples were examined for parasitic infestations and found negative. The hematological parameters were found within the normal range. Throughout the experiment, the Institute Animal Ethics Committee-approved ethical criteria for the correct care and management of experimental animals were adhered to.

2.2. Experimental design

The animals were divided into four equal groups. The animals in Group I were served as healthy control. Group II animals got flubendiamide (Fame, Bayer Cropscience Limited, Sabarkanta, Gujarat, India) @ 0.024 mg/kg/day orally for a period of 90 days. Group III animals got lead acetate (Merck Ltd., Worli, Mumbai, India) at 9.2 mg/kg/day. Animals in Group IV received both flubendiamide (@ 0.024 mg/kg/day) and lead acetate (@ 9.2 mg/kg/day) orally for a period of 90 days. Blood samples were collected by jugular venipuncture on 0, 30, 60, 90 days of treatment as well as day 30 after the end of the treatment. Serum was harvested and stored at −80°C till analysis.

Enzyme-linked Immunosorbent Assay (ELISA) kits from Monobind Inc., USA were used for the microplate competitive enzyme immunoassay (Type 7) to quantitatively determine the amounts of T3 and T4 in plasma. An enzyme immunoassay needed the following essential reagents: natural antigen, enzyme-antigen conjugate, antibody, and color-producing substrate. The native antigen and the enzyme antigen conjugate competed with one another for a restricted number of antibody binding sites when biotinylated antibody, enzyme-antigen conjugate, and serum containing the native antigen were combined. The streptavidin immobilised on the microwell and the biotin bound to the antibody underwent a synchronous reaction. This had an impact on how the antibody-bound fraction was separated following aspiration or decantation. The enzyme activity in the antibody bound fraction, determined by reaction with TMB (Tetramethyl benzidine), is inversely proportional to the native antigen concentration. A dosage response curve was created using multiple serum references with known antigen concentrations. Using this curve, the antigen concentration in the test sample was determined.

ELISA kits from Monobind Inc., USA were used for the immunoenzymometric test (Type 3) to assess plasma TSH activity. Excess natural antigen and high affinity

and specificity antibodies (enzyme conjugated and immobilised) with separate and diverse epitope recognition were necessary reagents for an immunoenzymometricassay. In this process, streptavidin coated in the well and exogenously supplied biotinylated monoclonal anti-TSH antibody interact to immobilise the sample during the experiment at the surface of a microplate well. When monoclonal biotinylated antibody, enzyme-labeled antibody, and serum containing native antigen were combined, there was no steric hindrance or competition between the antibodies and the native antigen, and a soluble sandwich complex was formed. The compound was simultaneously deposited into the well by the streptavidin and biotinylated antibody's strong affinity response. Decantation or aspiration was used to separate the antibody-bound fraction from the unbound antigen once equilibrium had been reached. The native antigen concentration was directly correlated with the enzyme activity in the antibody-bound fraction as determined by a reaction with tetramethylbenzidine. A dosage response curve was created using multiple serum references with known antigen concentrations, and it was from this curve that the antigen concentration in an unknown sample was calculated.

2.3. Statistical analysis

The initial tests performed on the data were Levene's and Kolmogorov-Smirnov tests for homogeneity of variance and normality. One-way or two-way analysis of variance (ANOVA) was used to assess differences between the control and various treatment groups using SPSS v13.0 (SPSS, Chicago, USA). $P< 0.05$ was regarded as significant, and the data were reported as the mean ± standard error (SEM). The association between each treatment group's blood Pb concentration and its T3, T4, andthyroid stimulating hormone (TSH) levels was ascertained using Pearson's correlation analysis.

3. Results

On days 60 and 90, the T3 level did not significantly decrease after receiving flubendiamide orally once a day (**Figure 1**; **Table 1**). On the other hand, T3 levels rose dramatically from day 0 and day 90 on day 30 of the treatment. Day 60 T3 level was substantially lower than in the group treated with lead alone. Nonetheless, there was no discernible difference between the T3 levels on various observation days and the comparable values in the control and other treatment groups. Day 90 post-treatment saw a substantial decrease in T4 level of 24.92%, and day 30 showed another marginally non-significant increase (**Figure 2**; **Table 2**). Only on day 90 the T4 level was significantly lower than the control. It did not deviate considerably from equivalent values in the control on other observation days. The TSH activity of animals treated with flubendiamide did not exhibit significant variation throughout observation periods; however, on day 90, the value was 6.5% lower than on day 0 (**Figure 3**; **Table 3**). Additionally, on day 90, the TSH activity was statistically equivalent to the control value but considerably lower than the corresponding levels in groups III and IV. Day 30 post-treatment activity increased by 15.62% over day 90 value; nonetheless, there was no meaningful difference between the two.

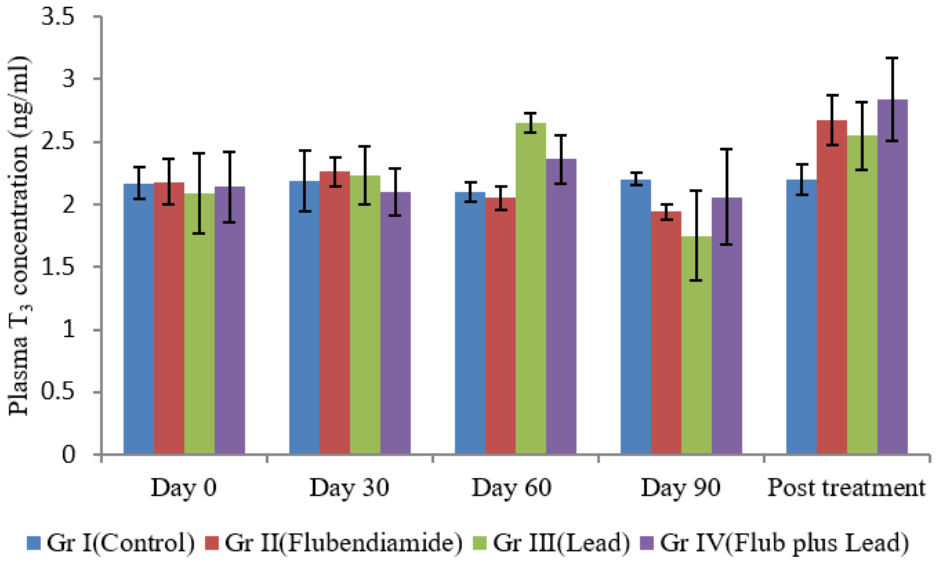

Figure 1. Changes in plasma T3 concentration in animals receiving flubendiamide and lead.

Table 1. Plasma T3 concentrations on different observation period.

Group	Day of observation				
	0	**30**	**60**	**90**	**30 post-treatment**
I	2.17 ± 0.13^{aA}	2.19 ± 0.24^{aA}	2.10 ± 0.08^{aA}	2.20 ± 0.05^{aA}	2.20 ± 0.12^{aA}
II	2.18 ± 0.18^{aA}	2.26 ± 0.12^{abA}	2.05 ± 0.09^{aA}	1.94 ± 0.06^{aA}	2.67 ± 0.20^{bA}
III	2.09 ± 0.32^{aA}	2.23 ± 0.23^{aA}	2.65 ± 0.08^{aB}	1.75 ± 0.36^{aA}	2.55 ± 0.27^{aA}
IV	$2.14 \pm 0.28^{a\,A}$	2.10 ± 0.19^{aA}	2.36 ± 0.19^{aA}	2.06 ± 0.38^{aA}	2.84 ± 0.33^{aA}

Note: Gr I: Control; Gr II: Flubendiamide; Gr III: Lead; GrIV: Flubendiamide + Lead. Values bearing different superscript in small letters in a row and capital letters in a column differ significantly ($P < 0.05$).

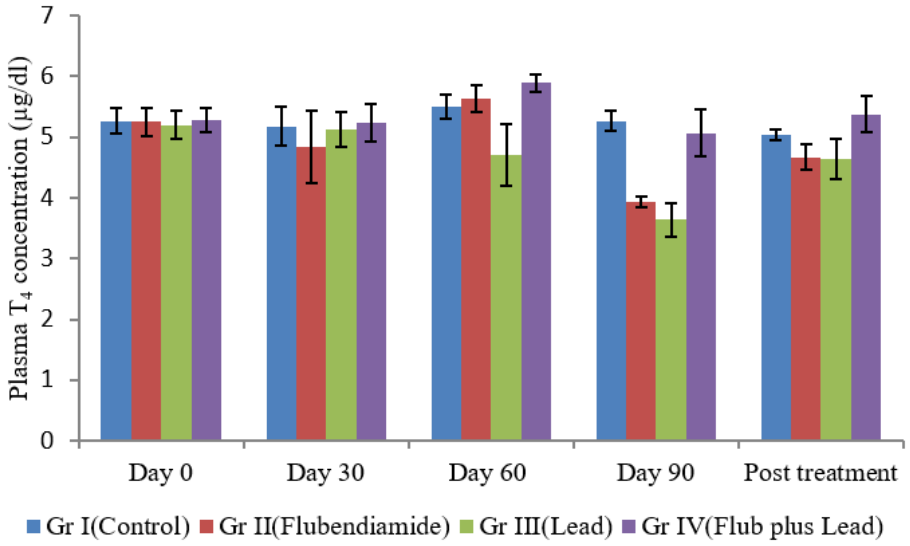

Figure 2. Changes in plasma T4 concentration in animals receiving flubendiamide and lead.

Table 2. Plasma T4 concentrations on different observation period.

Group	Day of observation				
	0	**30**	**60**	**90**	**30 post-treatment**
I	5.27 ± 0.22^{aA}	5.18 ± 0.32^{aA}	5.50 ± 0.19^{aAB}	5.27 ± 0.16^{aB}	5.04 ± 0.08^{aA}
II	5.25 ± 0.24^{bA}	4.84 ± 0.59^{abA}	5.63 ± 0.22^{bB}	3.94 ± 0.09^{aA}	4.67 ± 0.21^{abA}
III	5.20 ± 0.23^{bA}	5.13 ± 0.28^{bA}	4.70 ± 0.51^{abA}	3.64 ± 0.27^{aA}	4.64 ± 0.33^{abA}
IV	5.29 ± 0.20^{aA}	5.24 ± 0.31^{aA}	5.89 ± 0.15^{aB}	5.07 ± 0.39^{aB}	5.38 ± 0.29^{aA}

Note: Gr I: Control; Gr II: Flubendiamide; Gr III: Lead; GrIV: Flubendiamide + Lead. Values bearing different superscript in small letters in a row and capital letters in a column differ significantly ($P < 0.05$).

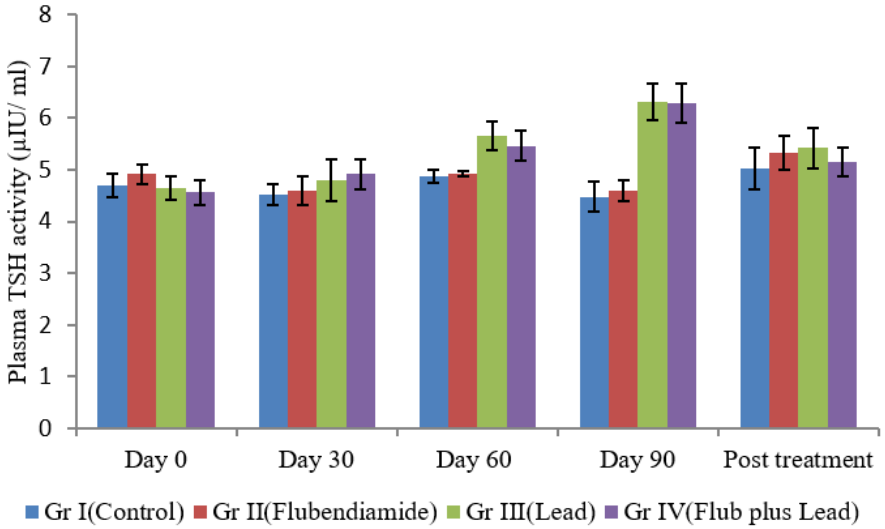

Figure 3. Changes in plasma TSH concentration in animals receiving flubendiamide and lead.

Table 3. Plasma TSH concentrations on different observation period.

Group	Day of observation				
	0	**30**	**60**	**90**	**30 post-treatment**
I	4.69 ± 0.23^{aA}	4.53 ± 0.20^{aA}	4.88 ± 0.12^{aA}	4.48 ± 0.30^{aA}	5.02 ± 0.41^{aA}
II	4.92 ± 0.19^{aA}	4.59 ± 0.28^{aA}	4.93 ± 0.05^{aA}	4.60 ± 0.21^{aA}	5.32 ± 0.33^{aA}
III	4.65 ± 0.22^{aA}	4.80 ± 0.41^{aA}	5.66 ± 0.27^{abB}	6.32 ± 0.35^{bB}	5.42 ± 0.40^{abA}
IV	4.56 ± 0.23^{aA}	4.92 ± 0.29^{aA}	5.46 ± 0.29^{abAB}	6.28 ± 0.38^{bB}	5.16 ± 0.28^{aA}

Note: Gr I: Control; Gr II: Flubendiamide; Gr III: Lead; Gr IV: Flubendiamide + Lead. Values bearing different superscript in small letters in a row and capital letters in a column differ significantly ($P < 0.05$).

On days 30 and 60, the T3 level in the animals administered lead acetate increased slightly; but, on day 90, there was a non-significant reduction (16.27 %) from the day 0 levels. Day 30 of the treatment saw another increase in the level, although there was no discernible difference in the readings throughout the observation periods. Furthermore, T3 levels, with the exception of day 60, when they were significantly higher than those of other groups, were statistically comparable to those seen in other treatment groups and the control group during various observation periods. T4 levels showed a falling trend with exposure length; however,

only the day 90 level was considerably (30%) lower than the day 0 value. On day 60, T4 level was significantly lower than all other groups and on day 90, it was significantly lower than control and group IV. Animals treated with lead showed a rising trend in TSH activity over time. Day 90 saw a 31.95% increase in activity over Day 0, a considerable increase. TSH activity peaked on day 90 compared to all other treatment groups, and on day 30 after treatment, there was a slight decline.

When rats were concurrently exposed to lead and flubendiamide, changes in their T3 levels were irregular and not statistically significant throughout different observation periods. The T3 level was somewhat lower (3.74%) on day 90, but it rose once more on day 30 of the treatment to a level 32.71% greater than day 0. During a certain observation period, there was no significant difference seen between the values of different treatment groups. Additionally, T4 level fluctuated non-significantly and inconsistently among observation days. Day 90 T4 level was 4.16% lower than day 0 value, but day 30 post-treatment level was 5.17% higher than day 0. On days 30 and 60, TSH levels slightly increased, although the differences were not statistically significant. The TSH level rose significantly on day 90, reaching a number that was 377.72% greater than the amount on day 0. TSH activity dropped to a level below the day 0 value on day 30 after therapy.

4. Discussion

The current study's findings showed that flubendiamide administration to male buffalo calves has thyrotoxic effects. The results of two unpublished investigations on the long-term toxicity of flubendiamdie were consistent with the present findings. For two years, rats were exposed to 34 to 44 mg of flubendiamide per kg of body weight, which caused thyrotoxic consequences [15,16]. Histopathological changes in flubendiamide-intoxicated rats included increased incidences of follicular cell hypertrophy with hydropic changes, increased large-size follicles and altered colloid. After being exposed to a dose ten times higher than that of male rats, female rats showed the same abnormalities in their thyroid ultrastructure. Therefore, it seemed that males were more vulnerable to flubendiamide's thyrotoxic effects [17].

Current observations were in agreement with another report recording a non-significant decline in T3and T4 levels in male Albino rats after lead-intoxication [18]. A pattern of low serum TSH and peripheral T4 levels in workers exposed to moderate to high amounts of lead has been reported in several investigations, indicating the possibility of secondary hypothyroidism. In a study on brass-foundry workers, low serum T4 with nearly normal TSH content was noted following significant lead exposure [19]. Another study on male car workers exposed to low levels of lead over an extended period of time found a negative association between blood lead levels and free T4, but not between TSH and T3 [5]. Similarly, another study found that automobile workers with high blood lead levels (52 µg/dL) had considerably greater TSH, but not significantly different T3 or T4 levels [20]. Conversely, Swarup et al. [21] found that cows raised near a polluted industrial area had much higher T3 and T4 levels, with a mean blood lead level of 0.51 µg/mL. In these cows, a strong positive association was found between the blood lead level and the concentrations of T3 and T4.

Lead has numerous harmful consequences, one of which is disruption of the pituitary-thyroid axis via an unidentified mechanism [3]. Thyroid indicators may exhibit an increased or inconsistent pattern at lower blood lead concentrations, but at higher blood lead concentrations (>50 to 98 µg/dL), they show a falling trend [22]. The response appears to vary with blood lead concentration. Lead's thyrotoxic effects are still unclear, despite a number of theories being put up. These include reduced thyroid gland iodine absorption [23] and changes in thyroxin metabolism or protein binding [19].

This study suggested thyrotoxic potential of flubendiamde in buffalo calves. However, further study is required to elucidate the mechanism of toxic action and also whether results obtained in the present study can be extrapolated in other mammalian species or not.

5. Conclusion

Based on the current investigation, it can be concluded that lead exposure alone or along with flubendiamide causes thyrotoxic consequences in mammals. The level of circulating thyroid hormone changes inconsistently when Pb and flubendiamide are exposed together, indicating that the interaction between the two toxicants and their effect on thyroid may vary with the dosage and/or length of exposure. To fully understand the thyrotoxic action mechanism of flubendiamide and the combined effects of these two thyrotoxicants, more research need to be undertaken.

Author contributions: Conceptualization, AR and VKD; methodology, AR, RR, VKD; software, AR; validation, VKD and RR; formal analysis, AR; investigation, AR; resources, VKD; data curation, AR, VKD and RR; writing—original draft preparation, AR and RP; writing—AR, RR and RP; visualization, VKD; supervision, VKD and RR; project administration, VKD; funding acquisition, VKD and AR. All authors have read and agreed to the published version of the manuscript.

Conflict of interest: The authors declare no conflict of interest.

References

1. Nishimatsu T, Hirooka T, Kodama H, et al. Flubendiamide: A new insecticide for controlling lepidopterous pests. In: Proceedings of the BCPC International Congress, Crop Science & Technology;31 October–2 November 2005; Glasgow, UK. pp. 57-64.
2. Tohnishi M, Nakao H, Furuya T, et al. Flubendiamide, a novel insecticide highly active against Lepidopterous insect pests. Journal of Pesticide Science. 2005; 30(4): 354-360.doi: 10.1584/jpestics.30.354
3. Pearce EN, Braverman LE. Environmental pollutants and the thyroid. Best Practice and Research Clinical Endocrinology and Metabolism. 2009; 23(6): 801-813.
4. Lopez CM, Pineiro AE, Nunez N, et al. Thyroid hormone changes in males exposed to lead in the Buenos Aires area (Argentina). Pharmacological Research. 2000; 42(6): 599-602.
5. Dundar B, Oktem F, Arslan MK, et al. The effect of long-term low-dose lead exposure on thyroid function in adolescents. Environmental Research. 2006; 101(1): 140-145. doi: 10.1016/j.envres.2005.10.002
6. Spieler RE, Weber DN. Effects of waterborne lead on circulating thyroid hormones and cortisol in rainbow trout. Medical Science Research. 1991; 19(15): 477.
7. Chaurasia SS, Kaur A. Protective effects of vitamin E against lead-induced deterioration of membrane associated type-I iodothyronine 5'-monodeiodinase (50 D-I) activity in male mice. Toxicology. 1997; 124(3): 203–209.

8. Bhaskar V, Dumka VK. Hematological alterations on sub-acute exposure to Flubendiamide in Sprague Dawley rats. Toxicology International. 2014; 21(3): 288-293.
9. Ranjan A, Dumka VK, Singh ND. Effect of flubendiamide, lead and their combined exposure on erythrocytic indices in water buffalo calves. Bulletin of Environmental Contamination and Toxicology. 2014; 92(4): 410-414. doi: 10.1007/s00128-013-1167-4
10. Ranjan A, Dumka VK, Ranjan R. Alterations in leukogram of buffalo calves following oral administration of flubendiamide, lead and theeir combination. Indian Journal of Veterinary Medicine. 2016; 36(2): 103-106.
11. Ranjan A, Dumka VK, Ranjan R. Chronicflubendiamide exposure induces oxidative stress in water buffalo (Bubalus bubalis). Current Science. 2018; 114(8): 1610-1612.
12. Venkatachalam D, Dumka VK. Pharmacokinetic profile of cefquinome after oral subchronicflubendiamide exposure and in vitro plasma protein binding in buffalo calves. Environmental Toxicology and Pharmacology. 2015; 39: 321-326. doi: 10.1016/j.etap.2014.12.004
13. Kortenkamp A, Altenburger R. Synergisms with mixtures of xenoestrogens: A reevaluation using the method of isoboles. The Science of Total Environment. 1998; 221: 59-73.
14. Kortenkamp A. Ten years of mixing cocktails: A review of combination effects of endocrine-disrupting chemicals. Environmental Health Perspective. 2007; 115(Suppl 1): 98-105.
15. Enomoto A. NNI-0001: Repeated dose 1-year oral toxicity study in rats: Final report. Institute of Environmental Toxicology, Japan; 2004; p. 986. Unpublished work.
16. Takeuchi Y. NNI-0001: Carcinogenicity study in mice: Final report. Institute of Environmental Toxicology, Japan; 2004; p. 648. Unpublished work.
17. APVMA. Public release summary on evaluation of the new active flubendiamide in the products BELT 480 SC insecticides and BELT 240 WG insecticide. Australian Pesticide and Veterinary Medicines Authority; 2009. Available online: https://www.apvma.gov.au/sites/default/files/publication/13766-prs-flubendiamide.pdf (accessed on 12 October 2024).
18. Ibrahim NM, Eweis EA, El-Beltagi HS, et al. The effect of lead acetate toxicity on experimental male albino rat. Asian Pacific Journal of Tropical Biomedicine. 2012; 2(1): 41-46.
19. Robins JM, Cullen MR, Connors BB, et al. Depressed thyroid indexes associated with occupational exposure to inorganic lead. Achieves of International Medicine. 1983;143(2): 220-224. doi: 10.1001/archinte.1983.00350020038008
20. Singh B, Chandran V, Bandhu HK, et al. Impact of lead exposure on pituitary-thyroid axis in humans. Biometals. 2000; 13: 187-192.
21. Swarup D, Naresh R, Varshney VP, et al. Changes in plasma hormones profile and liver function in cows naturally exposed to lead and cadmium around different industrial areas. Research in Veterinary Science. 2007; 82(1): 16-21.
22. ATSDR. Toxological Profile for Lead. Agency for Toxic Substances and Disease RegistryDivision of Toxicology and Environmental Medicine/Applied Toxicology Branch, Georgia.2005. Available online: https://www.atsdr.cdc.gov/toxprofiles/tp13.pdf (accessed on 12 October 2024).
23. Katti SR, Sathyanesan AG. Lead nitrate induced changes in the thyroid physiology of the catfish Clarias batrachus (L). Ecotoxicology and Environmental Safety. 1987; 13(1): 1-6.