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Original Research

Lead induced thyroid dysfunction in Wistar albino rats and its amelioration with *Ocimum sanctum* leaf extract – a hormonal and histopathological study

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Abstract

The objective of this study was to determine the effect chronic administration of different doses of lead acetate and study the role of Ocimum sanctum in lead toxicity as ameliorating agent in wistar rats. In the present investigation rats were randomly divided into six groups (n=36). Rats of group II and III were treated with different doses of lead acetate (60 mgs and 30 mgs / Kg.b.wt. / 3days a week respectively) and *Ocimum sanctum* orally @ 400mg/Kg b.wt. for other IV and V groups of rats along with lead acetate over a period of 12 weeks and the function of thyroid gland was evaluated by measuring the levels of serum thyroid hormones T3, T4 and TSH and histopathological changes of were studied in thyroid organ at every fortnight interval. Results in the present study revealed that lead acetate caused a dose dependant reduction in T3, T4 and TSH levels when compared to control. Hisopathologically thyroid shows hemorrhages and sever desquamation of epithelial cells, complete absence of acinar colloid in majority of acini, disruption of acini, atrophy of acini and interacinar fibrous tissue proliferation in majority of the animals. In *Ocimum* treated groups the various thyroid hormonal levels were significantly improved and histopathological changes in thyroid gland was mild when compared to lead treated rats. Based on changes it was concluded that lead acetate at 60 mg/ Kg b.wt. and 30 mg/ Kg b.wt. for 12 weeks was toxic to rats and effect of OS in higher lead dose group was minimum and amelioration was effectively observed in lower dose levels of lead acetate.

INTRODUCTION

Lead that contaminates the environment is largely air – borne but is redeposited by dust in to soil and water and is taken up by or exists on the surface of plants which are grazed by livestock [1]. The absorbed lead is conjugated in the liver and passed to the kidney where a small quantity is excreted in urine and rest accumulates in various body organs and interferes with their function. Lead can cause profound hematological, neurological, gastrointestinal, renal rheumatological and endocrine manifestations in man even at levels previously considered safe [2]. Lead exposure also causes functional impairment of pituitary – adrenal axis [3]. The thyroid gland plays an important role in energy usage, synthesis of RNA protein, consumption of oxygen by cells, overall body metabolism, growth processes and neurological development[4].Serum levels of thyroid hormones, including T3, T4 and TSH, are commonly used as reliable indicators of the thyroid function in humans and experimental animals. Changes in the serum concentration of these hormones can reflect disturbance in their glandular synthesis and secretion as well as disorders in their extra thyroidal peripheral metabolism [5].

Several authors tried various ameliorating agents like Thiamine, vitamin E, selenium, zinc etc. There was a meager information was available regarding herbal products as ameliorating agent. Keeping in view, *Ocimum sanctum* was used as an ameliorating agent in present research. *Ocimum sanctum* (OS) commonly known as 'Tulasi' in Hindi is a medicinal plant commonly grown in India. The use of this herb has been reported in Indian traditional systems of Medicine and its modern applications are receiving wide spread attention day by day. Different parts of this plant have been claimed to be valuable in a wide spectrum of diseases. It has been observed that tulasi has antioxidant, antibiotic, antiatherogenic, immunomodulatory, anti-inflammatory, analgesic, antiulcer, chemopreventive and antipyretic properties[6].

The literature regarding effect of lead on thyroid function in animals was very meager. Hence the present study was undertaken to study the toxic effect of lead on thyroid gland and amelioration with *Ocimum sanctum*.

MATERIALS AND METHODS

Studies were conducted on healthy adult male Wistar rats weighing more than 150 g. All the rats were housed comfortably in standard rat cages at $25^{o} \pm 1^{0}$ C and a 12:12 hour interval light / dark cycle throughout the experimental period of 12 weeks and provided *ad libitum* feed and water. The approval of the institutional animal ethics committee permission was obtained prior to commencement of the experiment. After 10 days of acclimatization the animals were randomly divided into six groups. The Lead acetate ($(CCH_3\ COO_2)_2$ Pb $3H_2O$, M.w = 379.33) with a laboratory reagent grade was procured from the Qualigens Fine Chemicals, Bombay and *Ocimum sanctum* (OS) a leaf extract from the Natural Remedies Pvt. Ltd. Bengaluru, India.

Total of 216 healthy young male rats were randomly assigned to the control and treatment groups. Six groups of rats consisting of 36 rats in each group were used for the study. The lead acetate dose was selected based on the pilot study and OS dose was selected based on the observations of [7] who reported that 100, 200,300,400 mg /kg bwt of OS were administered to Wistar rats followed by intra peritoneal injections of DMBA (7, 12, Dimethyl Benz (a) Anthracene) (30mg /kg bwt) 90 minutes after the final dose of the extract. At this dose of DMBA induced the genotoxicity by formation of micronuclei. The animals pretreated with OS @ a concentration of 300mg and 400mg /kg bwt was significantly reduced micronuclei formation i.e. reduced genotoxic effect of DMBA. Hence the higher dose of OS i.e. 400mg /kg bwt was selected as dose.

Lead acetate was given orally after mixing in distilled water to rats at the dose rate of 1/10 LD_{50} (60 mg/ Kg b.wt/3 days a week) and 1/20 LD_{50} (30 mg/ Kg b.wt/3days a week) respectively to the groups II and III. In addition to lead acetate, *Ocimum sanctum* was given orally at a dose rate of 400 mg/ Kg b.wt. to the groups IV and V. Group I and VI were kept as DW control and *Ocimum sanctum* control. Rats from each group were randomly sacrificed at fortnight intervals after starting the experiment *i.e.*, 2^{nd}, 4^{th}, 6^{th} 8^{th}, 10^{th} and 12^{th} weeks necropsy done and thyroid was collected for histopathological examination. Tissue pieces were processed by routine paraffin embedding method and stained with hematoxyline and eosin. Serum samples were collected from all groups at each sacrifice and were used for the estimation of T_3, T_4 and TSH using kit obtained from UBI (United Biotech inc).

RESULTS

The mean serum T_3 (Tri Iodo Thyronine) values were 1.17, 0.87, 0.97, 0.91, 1.09 and 1.19 (n mol/L) in Group I to VI respectively and are given in Fig.1. There was a dose dependent significant ($P<0.05$) decrease in T_3 values of lead fed groups when compared to control. No significant difference was observed in between lead treated groups. Where as significant improvement ($P<0.05$) in T_3 values were recorded in ameliorated group of lower dose (Group V) when compared to Group III.

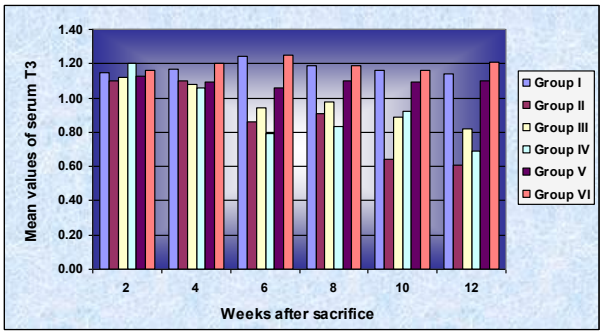

Fig.1. Mean values of serum T3 (n mol/L) in animals of different experimental groups

Statistically, a significant decrease in T_4 (Tetra Iodo Thyronine) values of lead treated groups (Group II and III) when compared to control (Group I). The details of data presented in Fig.2 and the mean T_4 values were 67.00, 27.83, 35.66, 31.16, 52.5 and 69.33 (n mol/L) in Group I to VI respectively. In between lead treated groups (II & III) a dose dependent significant ($P<0.05$) difference was noticed. No significant difference among group II (Higher dose) and group IV (ameliorated group of higher dose).Where as significant ($P<0.05$) improvement in T_4 values were observed in ameliorated group of lower dose (Group V)

when compared to lead treated group of lower dose (Group III).

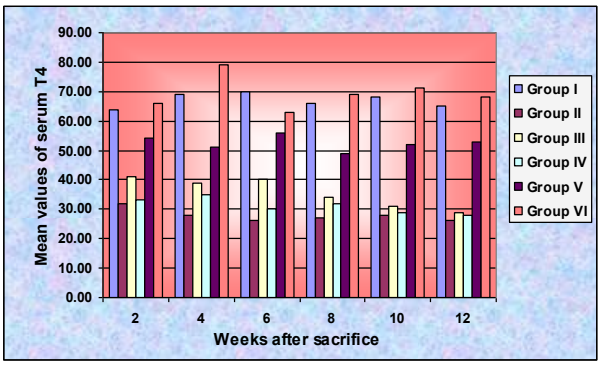

Fig.2. Mean values of serum T4 (n mol/L) in animals of different experimental groups

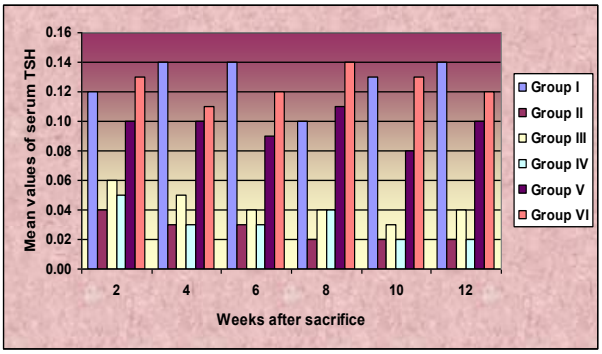

Fig.3. Mean values of serum TSH (m IU/L) in animals of different experimental groups

The mean TSH (Thyroid Stimulating Hormone**)** values of group I to VI were 0.12, 0.02, 0.04, 0.03, 0.09 and 0.2 (m IU/L) respectively and are given in Fig. 3. Statistically, a significant decrease in TSH values in lead treated groups was observed when compared to control animals (group I). Where as a significant difference was noticed among lead treated groups (II & III) and in between lead treated group of lower dose (group III) and its corresponding OS ameliorated group (Group V). No significant difference in TSH values in group II and IV.

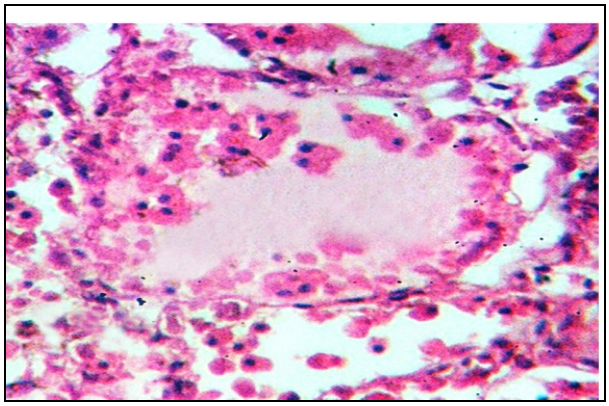

Fig.4.Thyroid: Group II: Section showing desquamation of acinar cells and absence of colloid in many acini. H & E: x 280.

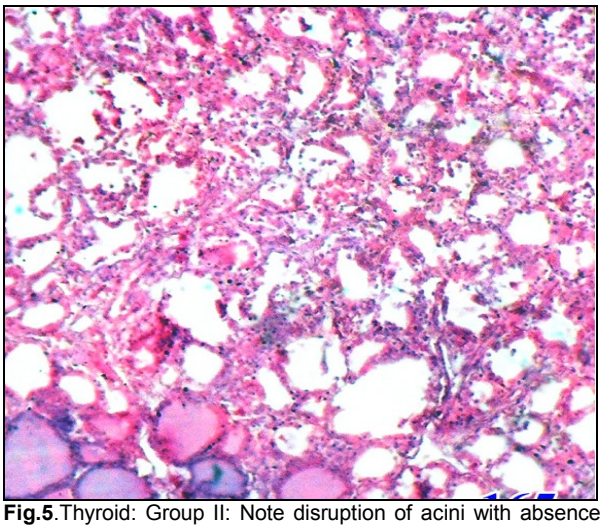

Fig.5.Thyroid: Group II: Note disruption of acini with absence of colloid. H & E: x 70.

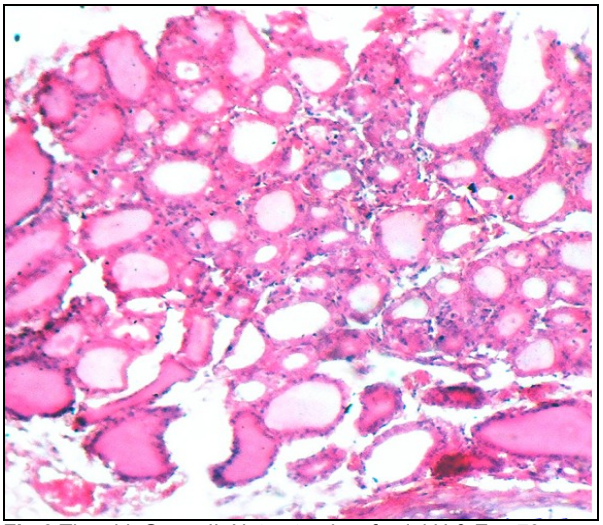

Fig.6.Thyroid: Group II: Note atrophy of acini.H & E: x 70.

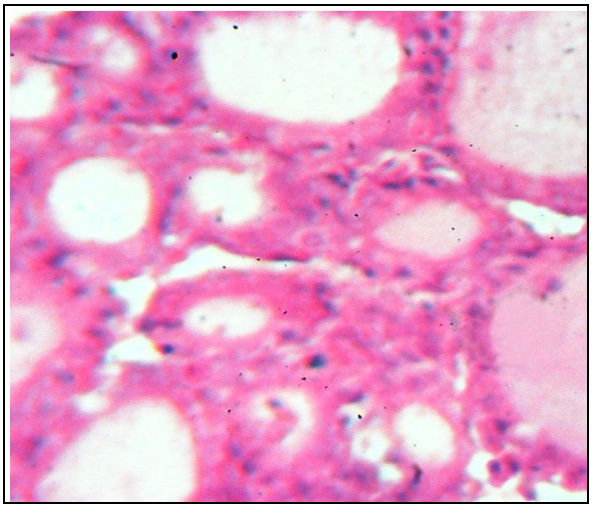

Fig.7. Thyroid: Group II: Note atrophy of acini.H & E: x 280.

Microscopically thyroid of majority of lead fed animals revealed hemorrhages and severe desquamation of acinar epithelial cells, complete absence of colloid (Fig.4) in majority of acini by the end of 2nd and 4th week. At places disruption of acini with absence of colloid (Fig.5), atrophy of acini and inter acinar fibrous tissue proliferation (Figs. 6&7) were observed during rest of the experimental period. Where as in Group III, the above changes were noticed with mild intensity, in group IV these changes were reduced mildly and in group V the changes were very mild by the end of 12th week and the thyroid regained its normal appearance.

DISCUSSION

Serum levels of thyroid hormones, including T3, T4 and TSH, are commonly used as reliable indicators of the thyroid function in humans and experimental animals All reactions necessary for the formation of T3 and T4 are influenced and controlled by pituitary, Thyroid stimulating hormone which stimulates follicular cells in the thyroid gland[5]. In the present study, a significant decrease in the serum T_3, T_4 and TSH levels were noticed in both lead fed groups (Group II and II) when compared to control in a dose dependent manner. In OS treated groups significant improvement was observed in lower dose group (group V) when compared to the III group. No significant improvement was noticed in group II and IV. The present results were in agreement with [8]. Hameed *et al.* [9]observed no significant alterations in T_3 and T_4 levels in Baladi goats fed with lead acetate @ 4.5 mg/kg bwt and 6 mg/kg bwt/orally from the beginning of pregnancy period to till the abortion. Similarly Vyskocil *et al.* [10] did not observe significant change in thyroid hormone levels in rats and petrol pump workers respectively. In contrary, increased thyroid hormone levels were observed by Dursun and Tutus [11] in occupationally exposed humans. The decrease in T3, T4 and TSH values might be due to structural damage of thyroid follicular cells might be due to accumulation of lead in the thyroid gland and also effect on regulatory enzymes associated with hypothalamic pituitary thyroid (HPT) axis [3].

In OS treated groups significant improvement was observed in lower dose group (group V) when compared to the group III. No significant improvement was noticed in group II and IV. This might be due to antioxidant property of OS [7].

In present study, microscopically thyroid of majority of lead fed rats revealed hemorrhages and sever desquamation of epithelial cells, complete absence of acinar colloid in majority of acini, disruption of acini, atrophy of acini and interacinar fibrous tissue proliferation in majority of the animals. This might be due to accumulation of lead in cells and damage cell membranes and disorders of the oxido – reductive processes in the cells [12]. In *Ocimum* treated groups these changes were mild when compared to lead treated groups. No reports were available to compare these results. This might be due to antioxidant property of OS [7].

The observations made in this study indicate that lead damages the structure and function of the thyroid gland. We suggest that the mode of action of mechanism of lead were by interference in the synthesis and / or secretion of T3, by the damage of thyroid follicular cells, decrease transformation rate of the T4 to T3 in peripheral tissue by inhibit the activity of type – I iodothyronine 5' - monodeiodinase(5' D) and interference with pituitary gland or hypothalamus gland. Severity of the disturbances increases with the time and dose of exposure. Amelioration with Ocimum sanctum significantly reduces toxicity changes in group V (lower lead dose) and a non significant improvement was noticed in group IV (higher lead dose) when compared to lead treated animals. For further studies suggest that the molecular mechanism of lead on thyroid gland and the Ocimum sanctum dose may be increased for reduction of lead toxicity changes at cellular level.

Table 1. Mean ±S.E values of serum T3, T4 & TSH in animals of different experimental groups

Weeks after sacrifice	Group I	Group II	Group III	Group IV	Group V	Group VI
serum T3 (n mol/L)	1.17^{a} ±0.01	0.87^{d} ±0.08	0.97^{bcd} ±0.04	0.91^{cd} ±0.07	1.09^{ab} ±0.00	1.19^{a} ±0.01
serum T4 (n mol/L)	67.00^{a} ±0.96	27.83^{e} ±0.90	35.66^{c} ±2.06	31.16^{de} ±1.07	52.5^{b} ±0.99	69.33^{a} ±2.23
serum TSH (m IU/L)	0.12^{a} ±0.00	0.02^{e} ±0.00	0.04^{cd} ±0.00	0.03^{de} ±0.00	0.09^{b} ± 0.00	0.2^{a} ±0.00

Mean ±S.E values with different superscripts differ significantly (P < 0.05)
ANOVA
S.E – Standard error

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