AGPE The Royal Gondwana Research Journal of History, Science, Economic, Political and Social Science
ISSN (P): 2582:8800 | A Peer reviewed | Annually | Open Accsess & Indexed
Volume 02 | Issue 01 | July 2021 | *www.agpegondwanajournal.co.in* | Page No. 31-38

ANILINE INDUCED HISTOLOGICAL ALTERATIONS AND EVALUATION OF SERUM HORMONES IN THYROID GLAND OF MALE ALBINO RATS

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Abstract:

Aniline is a primary aryl amine in which an amino functional group is substituted for one of the benzene hydrogen. It is a primary aryl mine and a member of anilines appears as yellowish to brownish oily liquid with a musty odour. Aniline has diverse uses in industry and in pharmaceuticals. It is used in rubber accelerators and antioxidants dyes and pigments fibres, photographic chemicals. It is also used in herbicides and fungicides. The objective of the present study is to investigate the effect of aniline on thyroid gland in albino rats. Eighteen adults albino rats were divided into three groups A, B and C. Group A was control and provided with normal food and water as well as Group B and C were received 20 mg/kg bw of aniline daily for 15 and 30 days respectively. After the completion of treatment body and organs weight and histology of thyroid gland and evaluation of serum hormones were examined. Results showed that there is decrease in body weight and increase in organ weight in experimental group as compare to control group of animals. Histopathology of thyroid gland of treated rats showed increase in number of microfollicles, reduce colloid secretion, vacuolation in colloid, breakdown of capsular layer, follicular disruption, fusion of thyroid follicles and increase interfollicular space as compared to control. Hormonal analysis of thyroid gland of treated rats showed significant decreased in thyroid hormones Tri-iodothyronine (T_3), Tetra-iodothyronine (T_4) level and increase Thyroid stimulating hormone (TSH) level as compared to control. From the results, It can be conclude that the aniline has potential to exerts deleterious effect on structure and function of thyroid gland.

Key words: Aniline, thyroid gland, histology, hormones, albino rat.

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Introduction:

Rapid industrialization and improper discharge of industrial effluents, wastes, accidental spills or deliberate release of certain hazardous chemicals that are mutagenic, carcinogenic and recalcitrant, pose a serious threat to environment including soils, groundwater as well as open water bodies.[1] These effluents have a variety of unusual chemicals including a range of aromatic hydrocarbon and their derivatives[2] which the microbes enzymatically decompose and utilize in cellular metabolism.[3]

Aniline is the aromatic amine and intermediate in chemical products of synthetic plastic, rubber, dye, medicinal drug, agricultural pest and clothing industries. Due to all that purpose it comes in water, air and finally goes in the human body workers who work in industry with aniline.[4] It (aminobenzene) serves as that parent compound for a common group of chemical intermediate, the halogenated anilines. Haloanilines are widely used in the manufactures of antioxidant, dyes, pharmaceuticals, agricultural chemicals and many products.[5] Aniline are the organic compounds which are also called amino benzene or phenyl amine.

Several studies have been demonstrated the chronic toxicity and carcinogenicity of aniline. In mammalian cell cultures, aniline had some mutagenic activity in mouse cells. Aniline is toxic by inhalation of the vapour, ingestion, or percutaneous absorption. The acute exposure of humans to elevated concentrations of aniline produces cyanosis, headache, nausea, vomiting, tachycardia, ataxia, vertigo, tinnitus, weakness, confusion, drowsiness, convulsions, coma, and death, primarily from the development of anoxia caused by methemoglobinemia.[6]

The thyroid gland, is an endocrine gland present in the neck, consisting of two lobes, left and right connected by an isthmus. It is butterfly-shaped organ found at the front of the neck, below the adams apple. The thyroid gland secrete thyroid hormones triidothyronine (T3) and tetraidothyronine (T4) which primarily influence the metabolic rate and protein synthesis. The hormones also have many other effects including those on development. The thyroid also produces the hormones calcitonin, which play an important role in calcium homeostasis.

According to the evidence found in the literature, aniline and its derivatives para-aminobenzoic acid (PABA) and 4,4 Diaminodiphenylmethene (DDPM) are biotoxic environmental pollutant widely present in natural food such as milk, eggs, brown rice and liver, but its daily requirement in humans in almost supplied by intestinal bacteria, [7] Somestudy showed that there derivatives causes inhibit induction of tumors also reported to cause goiter.[8]

According to [9] it is clearly indicate that PABA exerts progression effects on rat thyroid carcinogenesis followed by hypothyroidism and negative-feedback via the thyroid-pitutary axis. The histological study of the thyroid glands in the rats treated with DDPM showed diffused hyperplasia consist mainly of smaller follicles than those in the thyroid glands of untreated rats in DDPM-treated rats the follicle were lined with a layer of swollen cuboidal follicular epithelium.[10] Aniline and its derivatives showed effects on thyroid hormone. The rats treated with DDPM for 19 weeks showed lower serum concentration of tetraidothyronine (T4) and triidothyronine(T3) and higher thyroid weights than control rats this indicates that DDPM had goiterogenic effect. [10]

Less literature is available on effect of aniline on thyroid gland with short duration of albino rat. The purpose of the present work is to examine the effect of aniline on thyroid gland of albino rats and the following objectives were studied. Body weight changes pre and post treatment. Thyroid gland weight response after treatment, histological alteration in the thyroid gland in low dose with different duration. Analysis of thyroid hormones after treatment.

Materials and Methods:

Animal collection and maintenance: Eighteen adult male albino rats weighing between 253-271 grams were brought from the animal house unit in department of biochemistry, RTM Nagpur University Nagpur. The experimental protocol was approved by Institutional Animals Ethical Committee (IAEC) and animal care was taken as per the guidelines of Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA), Govt. of India (Registration No. 478/01/aCPCSEA). The rats were fed with pellet commercial diet daily and water was provide regularly.

Treatment: The animals were divided into 3 groups A, B and C, each group containing 6 animals. Group A was used as control and provided only with distilled water. Aniline 20 mg/kg bw administrated daily in group B and C for 15 and 30 days respectively. At the end of the treatment, animals were sacrificed by using anaesthetic Chloroform. At autopsy, body weight of each animal was recorded and thyroid gland were removed, cleaned, weighed and processed for histology.

Histology: After the completion of treatment, animals were sacrificed; thyroid gland of control and experimental animals were removed fixed in Bouin's fliud for 24 hrs. Then dehydrate by passing through graded series of ethyl alcohol, clear in xylene, embeded in paraffin wax, blocks were prepared and sectioned serially at 5µm. For histological study the sections were stained with haematoxyline and eosin. The photomicrographs were taken with the help digital camera Nikon COOLPIX 8400 attached to the light microscopes Nikon Eclipse E200.

Evaluation of Serum Hormones: For the serum hormone analysis, the blood was collected by cardiac puncture with the help of disposable syringe in a non-EDTA tubes and send to Saraswati pathology lab and research center for hormonal assay, It was done by CLIA Maglumi 800-SPLRC-2019. Analysis of triidothyronine (T3) tetraidothyronine (T4) was done by Radio Immuno Assay (RIA) and TSH was investigated by Institute of Rural Management Anand (IRMA) technique.

Statistical Analysis: The data were statistically analyzed and expressed as mean ± SEM. Statistical analysis of the control and experimental values was done using student's 't' test with the help of graph pad calculator.

Results:

Evalution of body, thyroid gland weight : The body weight increases with the age in the control rats but in treated animal body weight was deceases in both treated groups but more obvious was observed in 30 days treated groups. Thus there was a dose dependant short and long duration decrease in body weight of treated rats when compare with the control which tends to gain weight beacause of distilled water administration. weight of the thyroid gland was increased in treated

animals receiving aniline for 15 days duration but the weight of thyroid gland was more increase as the duration extended for 30 days. There was no significant changes observed in length of thyroid gland of rat exposed to aniline for 15 and 30 days as compared to control (Table No. 1).

Histology of thyroid gland: The animal with 15 days treatment with aniline showed increase in number of microfollicles, reduce colloid secretion in the lumen of the follicles, vacuolation in the colloids and reduction in size of the follicles as compare to control (Fig.1. C and D). The histopathological alterations were more obvious in animal treated with aniline 20 mg/kg daily for 30 days. In this treated group of animals showed more increased in the number of microfollicles, breakdown of capsualar layer, increased in intrafollicular space, follicular distruption, fusion of thyroid follicle, reduction of colloid, fusion and breaking of follicular cell line (Fig.1.E and F).

Evalution of serum hormones: the result revealed that serum hormonal analysis of thyroid gland of treated rats for 15 and 30 days showed significant decreased in thyroid hormones triiodothyronine (T3), tetraiodothyronine (T4) and thyroid stimulating hormone (TSH) increase as compared to control (Fig. 2, 3 and 4).

Discussion:

In the present study, significantly decrease in body weight was obtained after 15 and 30 days duration. These results are agreement with 4,4'- diaminodiphenylmethane (DDPM) [10], 4,4'-Methylene BIS (N,N-dimethyl) M-benzamine (MDBA) by[11]. In the study, the weight of thyroid gland was found to be increased in both treated group. These results are agreement with [10] treatment with DHPN+DDPM, DHPN, DDPM.[12]

The present study revealed that the thyroid gland of rats exposed to 20 mg/kg bw aniline for 15 days duration showed increasing the number of microfolicles, large follicles lined with follicular cells, slightly reduction of colloid amount and vacuolation in the lumen of follicles, increase follicular cell height, cytoplasmic vacuolation and follicular destruction. These results are in agreement with[10], [13][14] increased in epithelial cell height, significant reduction in size of the nucleus and change in nuclear shape from irregular,rounded, shrunken to ovule, elongated and pyknotic nuclei in rat thyroid treated with chromium.[15] showed the effect of propylthiouracil on thyroid gland and conferred the similar histological results.

In the present investigation the effect of 20mg/kg body weight of aniline on histology of thyroid gland for 30 days duration showed enlarged interfollicular spaces with reduced colloid in the follicles. These results are in agreement with [13] who studied the effect of chromium on thyroid gland showing enlarged interfollicular spaces with reduced colloid. In our study we also observed the vacuolation in the colloid, reduction in size of the follicle and disarranged nuclei of the follicular epithelial cells. [16] revealed the similar results with effects of high toxic dose of MSG on thyroid follicular cells of adult male albino rat showing loss of follicular pattern and follicular destruction, follicular hyperplasia and reduction incolloid.

In the present study serum hormonal analysis of male albino rats treated with 20mg/kg body weight of aniline showed significant decreases in thyroid hormone T3,T4 and increase in TSH as compared to control. These results are in agreement with [17]showed that serum T3 and T4 level

decrease significantly where as TSH level increase significantly in male and female rats treated for 4 days with 1.0 or 2.0gm/lit NaClO3 and for 21days with 2.0gm/lit NaClO3. [14] and for 21days with 2.0gm/lit NaClO3. [14] reported the similar result after the effect of PB and PTU which causes significant increase in TSH concentration and statistically decrease in T3 and T4 concentration.[18] also reported the increase in TSH and decrease in T3 and T4 level significantly after administration of NaF.

Legends:

Table 1. Body weight, organ weight and length of thyroid gland of albino rats of control and treated with aniline for 15 and 30 days duration. (values are expressed in Mean ± SE)

Fig. 1.A: T. S. of thyroid gland of control albino rat showing normal follicular cell (FC) with abundant colloid (CL) and peripherally positioned normal epithelial cell and there is interfollicular spaces (S) X40HE. B: T. S. of thyroid gland of control albino rat showing normal follicular cell (FC) with abundant colloid (CL) and peripherally positioned normal epithelial cell and there is interfollicular spaces (S) 400XHE. C: T. S. of thyroid gland of 15 days treated (aniline 20 mg/kg bw) albino rat showing large follicle (F) lined with follicular cell (FC) slight reduction of colloid with abundant colloid (RCL) amount and vacuolation (V) in the lumen of follicles, increasing number of microfollicles (MF) X40HE. D: Magnified view of fig.3 showing increase follicular cell height (ICH), cytoplasmic vacuolation (CV),follicular destruction (DF) X400HE. E: T. S. of thyroid gland of 30 days treated (aniline 20 mg/kg bw) albino rat showing increasing the number of microfollicles (MF), beakdown of capsular (C) layer increase intrafollicular spaces (IFC), blood vessels (BV) X40HE. F: Magnified view of connective tissue (CT), vacuolation (V), fusion of thyroid follicles (FU), follicular distraction (DF) and nucleus (N) X400HE.

Fig.2. Showing serum hormones level of triiodothyronine (T3) in control and treated rats for 15 and 30 days duration.

Fig.3. Showing serum hormone level of tetraiodothyronine (T4) in control and treated rats for 15 and 30 days duration.

Fig.4. Showing serum hormone level of thyroid stimulating hormone (TSH) in control and treated rats for 15 and 30 days duration

References:

1) Tani K., Mansuhara M., Welikala N., Yamaguchi N., and Nasu M (1998). Change in bacterial community during biodegradation of aniline. *J APPLMicrobiol*84:859-864.
2) Van der Meer J.R., Devos W.M., Harayamana S. and Zehnder A.JB (1992). Molecular mechanism of genetic adaptation to xenobiotic compounds. *Microbiol*(55):677-694.
3) Phale S.P., Basu A., Majhi D.P., Devery S. S., Vamsee K.C. and Shrivastava R (2007). Diversity in bacterial degradation of aromatic compounds. *J integrBiol* 11(3):252-279.
4) Benya T.J. and Cornish H.H. (1994). Aniline is patty industrial hygiene and toxicology. 4th edition, 982-984.
5) MonicaA.,Valentovic,JohnGB.,DianneK.,Anestis,KellyW.B.,ElioM.,John L.H. and Gary O.R. (1992). Acute renal and hepatic toxicity of 2-haloanilines in Fischer 344 rats. *Toxicology* (75):

121-131.

6) Gosselin R., Smith R.P. and Hodge H.C. (1984). Aniline. *clinical toxicology of commercial products, 5th Edition*

7) Altendorf K.H., Bachar A. and Lingens F. (1969). An intermediate involved in the formation of 4-aminobenzoic acid from chorismic acid in Aerobacteraerogenes. *FEBS* (3):319-321.

8) Fukushima S., Hirose M., Hagiwara A., Hagiwara R. and Ito N. (1981). Inhibitory effect of 4, 4'diaminodiphenyl methane on liver, kidney and bladder carcinogenesis in rats ingesting N-ethyl-N-hydroxyethylenenitrosamine or N-butyl-N-(4- hydroxybutyl)nitrosamine. *Carcinogenesis*(2):1033-1037.

9) Hasumura M., Imai T., Takizawa T., Ueda M., Onose J. and Hirose M. (2005). Promotion of thyroid carcinogenesis by para-aminobenzoic acid in rats initiated with N-bis (2-hydroxypropyl) nitrosamine. *Toxicological Science* 86(1)61-67.

10) Hiasa Y., Kitahori Y., Enoki N., Konishi N. and Shimoyama T.(1984).4, 4'-Diaminodiphenylmethene; promoting effect on the development of thyroid tumors in rats treated with N-Bis (2-hydroxypropyl) nitrosamine. *JNCL*, (72).

11) Weisburger E.K., Krishana Murthy A.S.,Lija H.S. and Lamb J.C. (1984). Neoplastic response of F344 rats and B6C3F1 mice to the polymer and dyestuff intermediate 4, 4'-methylenebis(N,N-dimethyl)-benzenamine, 4,4'-oxydianniline, and 4,4'-methylenedianiline. *JNCL*. 72(6).

12) Siglin J.C., Mattie D.R., Dodd D.E., Hildebrandt P.K. and Baker W.H. (2000). A 90- day drinking water toxicity study in rats of the environmental contaminate ammonium parchlorate. *Toxicological Science* (57):61-74.

13) Mohamad Z. and EI-Gharieb H. (2008).Effect of cadmium chloride on function of thyroid gland in rats. *Egypt J Comp Path and Clinic Pat* 22 (3):10-23.

14) Patil V.and Dhurvey V.T (2014). Exposere to sodium floride affects thyroid follicular cells in albino rats. Int. J of plant, animal and env. sci. vol. 5(1) - 56-61

15) Connor J.C., Frame S.R., Davis L.G. and Cook J.C. (1999). Detection of thyroid toxicants in a Tier 1 screening battery and alterations in thyroid endpoints over 28 days of exposure. *Toxicological Sciences*(51) 54-70.

16) Khalaf H. A. and Arafat E.A. (2015). Effect of different dosen of monosodium glutamate on the thyroid follicular cell of adult albino rats. A histological study. *Int J ClimExpPathol* 8 (12): 15498-15510.

17) HoothM.J.,DeangeloA.B.,GeorgeM.H.,GaillardE.T.,TravlosG.S.,Boorman G.A. and Wolf D.C.(2001). Subchronic sodium chlorate exposure in drinking water results in a concentration-dependent increase in rat thyroid follicular cell hyperplasia. *Toxicologic Pathology*29 (2) 250-259.

18) 18. Dhurvey V., Patil V., and Thakare M. (2017).Effect of sodium floride on the structure and function of the thyroid and ovaury in albino rats. (Rattus norvegicus). Fluoride. 50 (2):235-246.

Table 1

No. of animals	Treatment	BW at the beginning of the experiment (gm)	BW after treatment (gm)	Wt. of Thyroid gland (mg)	Length of Thyroid gland(cm)
6	Distilled water (15 days)	261.17 ± 1.92	264.0 ±1.75	13.50 ± 0.62	0.500 ± 0.026
6	Aniline (15 days)	262.83 ± 2.30	236.83 ± 1.96	15.00 ± 0.37	0.517 ± 0.031
6	Distilled water (30 days)	263.17 ± 2.06	269.83 ± 2.48	12.83 ± 0.60	0.500 ± 0.026
6	Aniline (30 days)	259.67 ± 2.99	210.50 ± 1.82	17.50 ± 0.43	0.533 ± 0.033

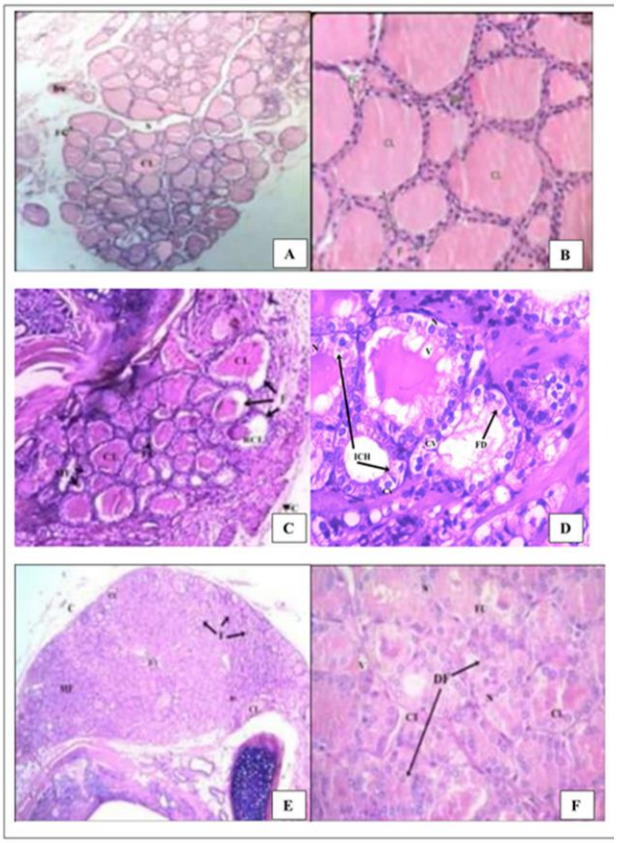

Fig.1

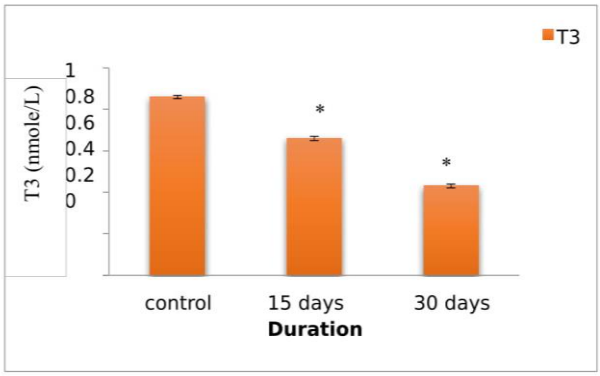

Fig.2

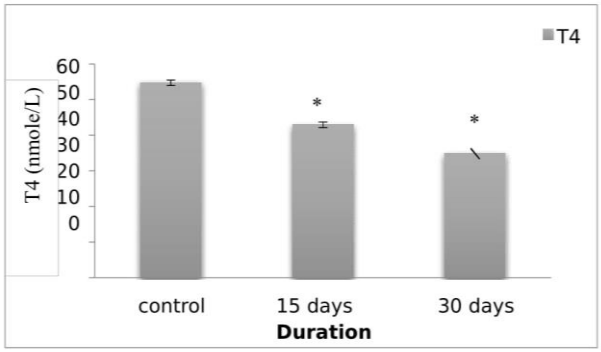

Fig.3

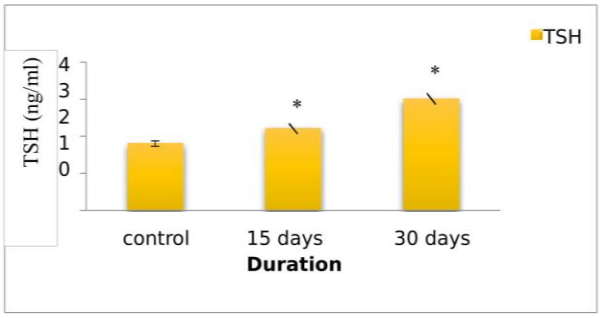

Fig.4

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