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AMELIORATIVE PROPERTIES OF *Pisonia alba* AGAINST ATRAZINE TOXICITY ON THYROID HORMONES ACTIVITY IN THE ALBINO WISTER RAT *Rattus norvegicus*

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Abstract

The present study was aimed to evaluate the effect of *Pisonia alba* against the toxicity effects of herbicide atrazine induced serum thyroid hormone T_3, T_4 and TSH in the Albino Wister rat *Rattus norvegicus*. The rats were intoxicated to sub-lethal dose of atrazine (0.25 mg of atrazine) for 28 days. The thyroid hormones T_3, T_4 and TSH were evidence by increased compared with the control. During the treatment of *Pisonia alba* against atrazine intoxicated rats were restored near normal level (Group II and III). The observed results were discussed in this present research paper.

Key words: Atrazine, *Pisonia alba*, *Rattus norvegicus* and Thyroid hormones.

1. Introduction

Atrazine is an herbicide primarily used on corn. Atrazine is the most common chemical contaminant of ground and surface water in the United States. It is a potent endocrine disruptor with ill effects in various animals and humans. Various studies suggested that an endocrine disruptor, an agent that may alter the natural hormonal system in animals (Hayes, *et al*., 2011). Currently, endocrine disruption induced by different chemicalsare one of the most important subjects in mammalian toxicology (Yuan *et al.*, 2009; Badraoui *et al*., 2010; Schug *et al.*, 2011).

The thyroid is one of the principal regulatory tissues of growth and development among multiple taxonomic groups which has been demonstrated to regulate diverse physiological endpoints including: metabolic rate, tissue differentiation and subsequent growth and development (Rousset and Dunn, 2004). The thyroid gland is located in the neck. It was most noted for the two major hormones are produces. These hormones are triiodothyronine (T_3) and thyroxine (T_4). Thyroid hormones are necessary for the normal development of body organs. A high concentration of thyroid hormones may change the metabolism of oxygen in the cells and stimulate the production of free radicals. The previous study demonstrated a significant decrease in mast cell number in the thyroid gland of juvenile/peripubertal male rats treated with atrazine at a 200 mg/kg bw dose, while the exposure to a ten times lower dose (20 mg/kg bw) had the same effect, but was not statistically significantwhen compared to the control (Rajkovic *et al*., 2010). The T_3 is the most metabolically active thyroid hormone (Kelly, 2000) and increases energy metabolism, heat production and thermogenesis (Silva, 1995; Kim, 2008).

Thyroid hormone formation involves a coordinated series of steps controlled by thyrotropin (TSH) but requiring insulin/insulin-

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like growth factor-1.Thyrotropin (TSH), the main secretory and growth stimulatory factor for the thyroid, is obviously involved in the production of H_2O_2 in that gland. However, atrazine was found stimulate triiodothyronine (T_3) and thyroxine (T_4) secretion in an acute experiment with treatment of rats (Ghinea *et al*., 1979). Tumor formation in the thyroid was well known to correlate with alteration in biochemical parameters like serum T_3, T_4 and TSH (Wilson *et al.*, 1996; Waritz*et al.*, 1996).

Pisonia grandis (Synonym: *Pisonia alba*) commonly known as Leechikottaikerai in Tamil, Velatisalet in Hindi (Khare, 2007). The plant *Pisonia grandis*, belonging to the family Nyctaginaceae, is an evergreen glaborous garden tree with young shoots are minutely puberulous. It is native of Hawai Island and naturalized throughout India. In the alternative system of medicine *Pisonia grandis* leaves are used as analgesic, anti-inflammatory, diuretic (Radha *et al*., 2008), hypoglycemic agent (Sunil *et al*., 2009) and antifungal (Shubashini and Poongothai, 2010). It is also used in the treatment of ulcer, dysentery and snake bite. The leaves are edible and mostly used to treat wound healing, rheumatism and arthritis (Prabu *et al*., 2008). Leaves also consumed as vegetable and salad, fed to cattle (Chatterjee and Prakashi, 1997). Herbal medicine is free from side effectsand less costly when compared to synthetic hypoglycemic agents. Nowadays, a lot of herbal formulations are available as tablets and other dosage forms. Hence, an attempt has been made to investigate the effect of administration of *Pisonia alba* extract on rats administered to a sub lethal dose of atrazine. Physiological parameters, including the values of serum thyroid hormone T_3, T_4 and TSH were chosen as specific indicators of animal response to the experimental treatments.

2. Materials and Methods

Experimental animal

Adult male albino Wistar rat (*Rattus norvegicus*) weighing (150 - 200 g) were obtained from Central Animal House, Rajah Muthiah Medical College (Reg No.160/1999/CPCSEA, Proposal number: 1096/2014), Annamalai University were used for the present investigation. The study protocol was approved by the Ethics Committee on AnimalExperiment, Faculty of Science, Annamalai University, Annamalai Nagar, Tamil Nadu, India.

Experimental chemical

Experimental chemical atrazine was purchased from (TATA Atrataf 50 % WP) manufactured by Rallis India Limited, Mumbai.

Preparation of Ethanolic extract

The dried powder was extracted (*Pisonia alba)* in a Soxhlet apparatus using ethanolat a temperature range of 55 °C to 60 °C. The filtrate was evaporated to dryness at reduced pressure in a vacuum evaporator.

Radioimmunoassay

The concentrations of thyroid hormones like T_3, T_4 and TSH in plasma were assayed by collecting blood from animals of all experimental groups at the end of the experiment (2 - 3 ml) by cardiac puncture under ether anesthesia. The RIA of TSH was carried out by using reagents obtained from NIDDK on credit from the USA National Hormone and Pituitary Program. Thyroid hormones in non-extracted plasma were assayed by using RIA T_3 and RIA Long-term bromide action on rat thyroid 15 T4 kits.

Experimental Design

A total of 24 animals will be divided into 4 groups of 6 in each.

Group 1: Control animals

Group 2: Atrazine alone (0.25 mg/kg bw)

Group 3: Atrazine (0.25 mg/kg bw) + *Pisonia alba* (1 ml/kg bw)

Group 4*:* *Pisonia alba* (1 ml /kg bw)

Statistical analysis

Results were expressed as mean ± S.E. Statistical analysis was performed using Student's t-test and p-values < 0.05 were considered statistically significant.

3. Results

Serum levels of T_3

The present study was aimed to investigate the serum levels of T_3 in *Rattus norvegicus* after atrazine administered was reduced in the group II when compared with the control group I. The percent changes were 4.240, 7.103, 7.2305, 6.784 and 6.765 for the period of 1, 7, 14, 21 and 28 days, respectively. While in the rat administered to atrazine along with *Pisonia alba* extract in group III was increased when compared with group II. The per cent changes were -3.543, -6.122, -5.979, -8.386 and -8.998for the period of 1 to 28 days, respectively. When extract of *Pisonia alba* alone administrated in group IV, the T_3 levels was decreased than in group III and it was near to control group. The percent changes were 1.231**,** 1.366, 1.500, 0.814 and 1.082 for the period of 1 to 28 days, respectively; the T_3 levels was increased than in group III and it was near to control group. The recorded T_3 levels in all the four groups were statistically significant at 1 % and 5 % levels.

Serum levels of T_4

In the present experimental observation showed that the serum levels of *Rattus norvegicus* after atrazine administered was reduced in the group II when compared with the control group I. The per cent changes were 7.082, 7.955, 8.304, 8.598 and 9.319 for the period of 1, 7, 14, 21 and 28 days, respectively. While in the rat administered to atrazine along with *Pisonia alba* extract in group III was increased when compared with group II. The per cent changes were 2.106, 1.085, 0.664, 0.112 and 0.846 for the period of 1 to 28 days, respectively. When extract of *Pisonia alba* alone administrated group IV, the T4 levelswas increased than in group III and it was near to control group. The per cent changes were -11.612, -11.295, -11.153, -10.835 and -10.129 for the period of 1 to 28 days, respectively. The recorded T_4 levels for all the four groups statistically significant at 1 % and 5 % levels.

Serum levels of TSH

In the present investigation showed that the serum TSH levels in the *Rattus norvegicus* after atrazine administered was significantly decreased in the group II when compared with the control group I. The per cent changes were -3.329, -3.287, -3.271, -3.708 and -4.328 for the periods of 1, 7, 14, 21 and 28 days, respectively. While in the rat administered to atrazine along with *Pisonia alba* extract in group III was decreased when compared with group II. The per cent changes were -0.923, -1.069, -1.069, -1.507 and -0.621 for the periods of 1 to 28 days, respectively. When extract of Pisonia *alba* alone administrated group IV, the TSH levels was decreased than in the group III and it was near to control group. The per cent changes were -13.006, -12.992, -13.027, -12.992and 13.099 for the period of 1 to 28 days respectively. The recorded TSH levels in Serum for all the four groups was statistically significant at 1 % and 5 % levels.

Table – 1: Changes of serumT_3 (ng/ml) levels in the liver of Albino Wister Rat *Rattus norvegicus* administered to atrazine followed by the extract of *Pisonia alba* exposed to 28 days

Groups		Treatment duration				
		1	7	14	21	28
T_3	Group-I Control	0`731±0.03	0.732±0.04	0.733±0.06	0.737±0.07	0.739±0.05
	Group-II Atrazine	0.762±0.13^{NS} 4.240	0.784±0.15^{NS} 7.103	0.786±0.16^{NS} 7.2305	0.787±0.16^{NS} 6.784	0.789±0.917^{NS} 6.765
	Group-III Atrazine + *Pisonia alba*	0.735±0.12^{NS} -3.543	0.736±0.04^{NS} -6.122	0.739±0.05^{NS} -5.979	0.721±0.04^{NS} -8.386	0.718±0.05^{NS} -8.998
	Group-IV *Pisonia alba*	0.740±0.05^{NS} 1.231	0.742±0.06^{NS} 1.366	0.744±0.06^{NS} 1.500	0.743±0.06^{NS} 0.814	0.747±0.08^{NS} 1.0825

Values are mean ± S.E - Mean of six individual observations and student t-test. Significant at *P<0.05; Significant at ** P < 0.01 levels, NS- Non-significant.

Table – 2: Changes of serum T_4 (µg/dl) activities in the Albino Wister rat *Rattus norvegicus* administered to atrazine followed by the extract of *Pisonia alba* exposed to 28 days

Groups		Treatment duration				
		1	7	14	21	28
T_4	Group-I Control	5.718±0.22	5.179±0.32	5.720±0.30	5.722±0.38	5.726±0.42
	Group-II atrazine % COC	6.123±0.31** 7.082	6.174±0.42 NS 7.955	6.195±0.34* 8.304	6.124±0.33 8.598	6.263±0.35** 9.319
	Group-III atrazine + *P. alba*	6.252±0.51 NS 2.106	6.241±0.32 NS 1.085	6.233±0.25 NS 0.664	6.221±0.23 NS 0.112	6.210±0.28 NS 0.846
	Group-IV *P. alba* % COC	5.054±0.21* -11.612	5.073±0.34** -11.295	5.082±0.32** -11.153	5.102±0.43** -10.835	5.146±0.43 NS -10.129

Values are mean ± S.E - Mean of six individual observations and student t-test. Significant at *P<0.05; Significant at ** P < 0.01 levels, NS- Non-significant.

Table – 3: Changes of serum TSH (µIU/dl) levels in the Albino Wister rat *Rattus norvegicus* administered to atrazine followed by the extract of *Pisonia alba* exposed to 28 days

Groups		Treatment duration				
		1	7	14	21	28
TSH	Group-I Control	7.058±0.42	7.058±0.43	7.062±0.33	7.064±0.32	7.069±0.45
	Group-II atrazine	6.823±0.43 NS -3.329	6.826±0.34 NS -3.287	6.831±0.60 NS -3.271	6.802±0.53 NS -3.708	6.763.±0.41 NS -4.328
	Group-III atrazine + *P. alba*	6.760±0.32 NS -0.923	6.753±0.42 NS -1.069	6.728±0.52 NS -1.069	6.724±0.33 NS -1.507	6.721±0.42 -0.621 NS
	Group-IV *P. alba*	6.140±0.32** -13.006	6.141±0.32** -13.027	6.142±0.42* -12.992	6.140±0.31* -13.027	6.143±0.31* -13.099

Values are mean ± S.E - Mean of six individual observations; and student t-test. Significant at *P<0.05; Significant at ** P<0.01 levels, NS – Non- significant.

4. Discussion

In the present study, the serum T_3, T_4 and TSH activities are estimated. The thyroid hormones, triiodothyronine (T_3) and its prohormone, thyroxine (T_4) are tyrosine - based hormones produced which are primarily responsible for regulation of metabolism. Iodine was necessary for the production of T_3 and T_4. A deficiency of iodine leads to decreased production of T_3 and T_4 enlarges the thyroid tissueand will cause the disease known as simple goiter. The major form of thyroid hormone in the blood was thyroxine (T_4), which has a longer half-life than T_3. The ratio of T_4 to T_3 released into the blood was roughly 20 to 1. The T_4 was converted to the active T_3 (three to four times more potent than T_4) with in cells by deiodinases (5'-iodinase).

Studies of Sadani *et al*. (1996) showed that the role of ROS and antioxidant defense of thyroid tissue in hyperthyroidism and hypothyroid goiter. Thyroid hormones have been reported to exhibit antioxidant properties (Bunyan *et al*., 1961; Faure *et al*., 1991). However, T_4 is the major hormone produced by the thyroid and most of them circulating T_3 (approximately 80 %) was formed from T_4 in peripheral tissues by the activity of type-I 5′-iodothyronine monodeiodinase (5′-DI) (Ko, 2000). When there was damage to the lipid membranes of the hepatocyte by the release of abnormal concentration of serum markers in to the blood stream. The increase may be the clear indicateof cellular leakage and loss at functional integrities of the cell membrane (Sarasvathi *et al*., 1993).

Atrazine did not influence thyroid carcinogenesis in this model, although atrazine was earlier reported to cause histological thyroid lesions along with dose dependent decrease of serum T_3 concentration (Kornilovskaya *et al*., 1996). Triiodothyronine (T_3) is a thyroid hormone. It plays an important role in the body's control of metabolism.The growth and proliferation in the

thyroid has not yet been satisfactorily explained. Both may be associated with the feedback mechanism in which the decrease in the level of thyroid hormones caused by iodine deficiency leads to an increased TSH secretion, which is important and well documented (Studer and Greer, 1965).

In previous studies atrazine delayed the onset of puberty and altered estrous cyclicity in the female Wister rat, but serum T_3, T_4 and TSH were unchanged and histopathology /morphologic lesions were lacking (Laws *et al*., 2000). All the possible mechanism of atrazine toxicity may lead to the formation of Reactive oxygen species (ROS) as found in present investigation. In an acute experiment with treatment, 50 ppm atrazine stimulated T_3, T_4 and synthesis (Ghinea *et al*., 1979). But, not mechanism underlying the thyroid effect of tamoifen was attributed to the modulation of thyroid peroxidase (Beyssen *et al*., 2000). Hormone concentration measured in rat serum on in toxic was compared with the control group values which, according to the results of previous studies were within the expected limits (Brown Grant *et al.,* 1970). Since, atrazine toxicity effect on a range ofcellular enzymes particularly those involved in energyproduction and associated with massive dilated mitochondria leading to hydropic degeneration appear as cytoplasmic vacuolization (Buchheim *et al.,*1998). There have been many reports of altered thyroid function with tamoxifen therapy, including suppression of plasma levels free T_3 (FT_3) and free T_4 (FT_4) and increased hence induction of hypothyroidism (Anker *et al*., 1998). However, tamoxifen has been reported to elevate T_3, T_4 and TBG (Gordon *et al*., 1986) or only TSH (Zidan and Rubenstein, 1999) in the serum of postmenopausal patients. The T_3 actions are mediated by binding to nuclear receptors (Oppenheimer *et al*., 1987). It must be noted that other iodothyronines such as 3, 5 diiodothyronine (T_2) have biological activities, including interfering with bioenergetic mechanisms (Goglia, 2005). In addition, lead acetate ingestion caused insignificantly reduction of plasma T_4 and T_3. There are in agreement with previous study, they found that Pb^{2+} decreased the Thyroxin (T_4) and the 3, 5- triiodothyronine (T_3) levels. This indicates that animals exposed damage (Yousif *et al*., 2009). However, this mechanism may not be the only one (Gaitan and Dunn, 1992) and growth factors such as immunoglobulin - stimulating thyroid growth factor, epidermal growth factor, insulin - like growth factors and transforming growth factor (Wenzel and Bottazzo, 1987) may also play a role. Gestational BDE-99 exposure produced alterations in TSH, T_3 and T_4 levels (Hakk *et al*., 2002; Kuriyama *et al*., 2007). In contrast, our data show that BDE-99 exposure of adult animals did not affectthese hormone levels.

A decrease in copper levels is correlated with changes in T_3 concentration, whereas T_4 was not altered. It has been often cited that a decrease in zinc levels influences thyroid hormone concentration mainly by decreasing T_3 concentration, whereas it does not influence T_4 (Kralik *et al.,* 1996). Finally, in another study, zinc supplementation in hepatotoxic rats lead to T_3 activity restoration, indicating an indirect effect of zinc on the regulation of thyroid hormone concentrations (Goel *et al*., 1994). These signs of thyroid malfunction may be the results of increased caspase-dependent apoptosis pathways (Esmekaya *et al*., 2010).

The present research work attempts to assess the importance of active compound of *P. alba* showed the presence of Vitamin A, Vitamin C, thiamine, riboflavin, nicotinic acid (Vitamin B3), alkaloids, proteins and fats. Vitamin C is one of the four dietary antioxidants, the others being Vitamin E, Vitamin A precursor β-carotene and Selenium. Flavonoids and phenolic acids have antibacterial, antifungal, antiviral, hepatoprotective, immunomodulation and anti-inflammatory properties and also play important role for regain the hormonal level of the rat. In the present investigation the atrazine treated group was increase the activity of T_3, T_4 and TSH. Moreover, the group IV (*Pisonia alba)* decreased the levels of these biochemical parameters. Because, the plant having such a wound healing property compounds like pinitol, apigenium, luteolin, chrysoeriol, vitamin E and rutin. But, the

group III (atrazine along with *Pisonia alba*) decreased activity was observed.

5. **Conclusion**

It was concluded that an increased in hormonal levels induces leads to the effects of atrazine. The alterations of these two trace elements have a direct influence on TSH release. On the other side, T_3, T_4 and TSH alterations are rather a result of wider influence of trace elements on several organs.The results indicate that the plant extract may become an important source of compounds with health protective potential. The plants also have more therapeutic properties.

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6. References

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