**Bulletin of Environment, Pharmacology and Life Sciences**
Bull. Env. Pharmacol. Life Sci., Vol 10 [11]October 2021 : 42-52

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

**ORIGINAL ARTICLE** **OPEN ACCESS**

# Marine organisms: Potential screening against methimazole induced hypothyroidism in rats

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**ABSTRACT**

*The study was aimed to evaluate the efficacy of marine organism extracts (n=10) in Methimazole (MTZ) induced hypothyroidism rats. The thyroid is one of the important and widespread persistent organic contaminants that accumulate in the food chain. It is assumed to cause endocrine-disrupting effects in animals, due to its possible transfer through marine food chains and to the consumption of contaminated seafood. The animals were separated into five groups such as control, MTZ treated (60mg/kg BW), MTZ + L-Thyroxine (0.5 mg/kg BW), and MTZ + Treated with marine organisms (100mg & 150mg /kg BW), are treated for 28 days. T3, T4, and TSH were estimated in serum, and SOD, Catalase, GPx, GST, and Lipid peroxidase were estimated in different tissues, the histopathological changes were observed by using H&E stain. Among 10 marine organism extracts TA1 of Spirulina platensis (150 mg/kg BW), has shown a significant (P < 0.05), effect on T3, T4, and TSH in blood serum levels compared to the untreated diseased group. The antioxidants such as superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), glutathione-s-transferase (GST), lipid peroxidase (LPx), and glutathione reduced (GSH) levels were significantly (p<0.01) decreased at the same time the levels of malondialdehyde MDA increased in MTZ-induced hypothyroidism rats. The results demonstrated that the TA1 150mg/kg BW shown an alleviative effect on the enzymatic and non-enzymatic antioxidants. On selected tissues, the histopathological changes showed that TA1 treatment had significant recovery in the organization of extracellular and cellular components. The S. platensis aqueous extract(TA1) has antioxidants activity which might help in the prevention of cellular and sub-cellular changes that occurs in the course of MTZ-induced hypothyroidism.*

**Keywords:** Hypothyroidism, marine organisms, Levothyroxine, Methimazole, *Spirulina platensis*.

Received 21.04.2021 Revised 06.07.2021 Accepted 09.09.2021

## INTRODUCTION

The hormones delivered by the thyroid gland regulates protein synthesis, use of oxygen, basal metabolic rate, the metabolic rate at a cellular rate, and overall growth and development. In India, a million people suffer from endocrine disorders. Several of these diseases are caused by environmental factors.[1] They are preventable and can be effectively treated at an affordable cost. Endocrine disorders are most often present with imprecise symptoms early in their diagnosis.[2] The full-blown syndrome emerges late, and by then several complications would have set in as a result of damage to vital organs.[3]

The hormones delivered by the thyroid gland regulates protein synthesis, use of oxygen, basal metabolic rate, the metabolic rate at cellular levels, and overall growth and development.[4]The thyroid hormones have their direct or indirect vital impact on various body functioning, including cardiovascular, respiratory, reproductive, metabolic, and neurological disorders.[5]The concentration of the pituitary glycoprotein hormone, thyroid-stimulating hormone transcendently directs the activity of the thyroid gland[6]. Two thyroid hormones, thyroxine (T4) and tri-iodothyronine (T3) are involved in the regulation of major body functioning. Measurement of the thyroid hormones T3 and T4 in the blood serum is viewed as a substantive assessment of thyroid capacity[7].

Hypothyroidism, specifically, is the most of thyroid disorder in India, affecting one in ten adults. It has been estimated that about 42 million of the population in India suffered from thyroid diseases.[8]Decreasing the levels of thyroid hormones leads to hypothyroidism. To date, hormone

replacement therapy is the most common strategy in the management of thyroid dysfunction. However, the therapy has not remained untouched from the possible side effects which include, cardiovascular changes, left ventricular hypertrophy, reduced bone density, skin rash, itching, and mild leukopenia. In a rare condition, the therapy may also cause aplastic anemia, thrombocytopenia-like syndrome.[9]Therefore, there is an unmet need for an alternative approach for the safe and effective management of hypothyroidism.

Marine microalgae or seaweeds are a large and heterogeneous group of photosynthetic organisms found in a marine environment[10], commonly classified into three taxonomic groups: brown algae (Phaeophyceae), red algae (Rhodophyta), and green algae (Chlorophyta).[11] Macroalgae usually part of the traditional food culture of many countries, where they have been cultivated on a large scale for centuries. In contrast to Asian countries, the exploitation of this resource in European countries has been very limited and mainly focused on the commercial manufacturing of thickeners e.g. agar and alginates[12]. Marine species makeup around half of the total global biodiversity and have been shown to possess many properties or activities such as antitumor, anti-diabetic, anti-thyroid[13]. This is due to the presence of oligosaccharides, glycolipids, phenolic compounds, and small molecular weight bioactive compounds, and their effect was proved in experimental animals[14]. Also, seaweeds especially species from Phaeophyceae may accumulate exceptional levels of iodine, which is well known to be an essential element for the maintenance of thyroid function and health.[15] Noted that iodine deficiency is a reality at least in 11 European nations and most of the remaining nations are using iodized salts to prevent this problem, the introduction of seaweeds in population eating habits could be a valid alternative to ensure intake of the optimal daily requirement of iodine[16]. Nevertheless, the usage of such marine organisms must have some precautions since the everyday intake of more than 600 µg of iodine tolerable intake level for adults may act in the opposite direction, causing poisoning effects[17]. Marine organisms are essential to the activity of certain enzymes (e.g., sulfite oxidase and xanthine oxidoreductase) that catalyze redox reactions, while Se is mainly present as part of selenoproteins, which have a variety of functions, including antioxidant effects, T-cell immunity, thyroid hormone metabolism, and skeletal and cardiac muscle metabolism.[18]Therefore our study was aimed to screen and identify the potential marine organism which shows high efficiency against methimazole (MTZ) induced hypothyroidism in albino rats

## MATERIAL AND METHODS

### Chemicals and reagents:

***Methimazole***(MTZ) Levothyroxine, Xylene, Dipyridyl, Ethanol, Tocopherol, Nitric oxide, butylated hydroxyl toluene (BHT), Thiobarbituric acid (TBA), tris base, ethylene diamine tetraacetic acid (EDTA), 5,5'- dithiobis-2-nitrobenzoic acid (DTNB), hydrochloric acid, dinitrophenyl hydrazine (DNPH), glutathione (GSH), trichloroacetic acid (TCA), NADPH, triton X-100, reduced glutathione (GR), oxidized glutathione (GSSG), nitro blue tetrazolium chloride (NBT), epinephrine, tetra butyl hydroperoxide, 1-chloro-2,4-dinitrobenzene (CDNB). All the chemicals are of analytical grade and procured from commercial local vendors.

### Collection of marine macroalgae, preparation of organic fractions of microalgae:

The marine organisms used in this study were *Chaetoceros calcitrans*(AM1), *Chlorella salina* (SD1), *Isochrysis galabana* (SD2)*, Spirulina platensis* (TA1)*, Sargassum wightii* (SS1)*, Nannochloropsis salina* (PV1), *Octopus membranaceus* (SK5)*, Crassostream adrasensis* (SK6)*, Sepia pharaonis* (SK7)*, Loligo duvauceli* (SK8)(Table-1) (Fig.1).The identities of the marine macroalgae and molluscan considered in the present study were ascertained with the sample specimens maintained in the Marine Biodiversity Museum of Central Marine Fisheries Research Institute. The marine macroalgae and molluscan species were collected freshly from the Gulf of Mannar in Mandapam region located between 8°48'N, 78°9' E and 9°14' N, 79°14' E on the southeast coast of India during the months spanning between August and April. Samples collected (2 kg) were washed in running water and shade dried before being pulverized to minimum particle size.

The microalgae belonging to *Chlorella salina, Tetraselmis tetrahele, Isochrysis galabana, Chaetoceros calcitrans,* and *Spirulina platensis* were isolated and cultured in the Mariculture Facility of CMFRI (Central Marine Fisheries Research Institute) (Fig. 1). The algal cells were counted using the hemocytometer chamber in an optic microscope. Culturing days were standardized based on the hemocytometer and UV spectrophotometer data. The cultured cells were harvested by centrifugation at 5000 rpm for 10 min at 4°C. The supernatant was decanted and the remaining biomass was washed in distilled water before freeze-drying. The freeze-dried samples were kept at -20°C until extraction. Lyophilized powders of the studied microalgae were extracted with organic solvents at 45°C for 4 h in a water bath before being

concentrated (44°C) in a rotary vacuum evaporator (Heidolf, Germany) to yield the concentrated crude extracts of microalgae.

**Table.1: List of marine organisms**

| Sl. No. | Name of the marine organisms | Species | Marine organism aqueous extracts code |
|---|---|---|---|
| 1. | *Chaetoceroscalcitrans* | *Algae* | AM1 |
| 2. | *Chlorella salina* | *Algae* | SD1 |
| 3. | *Isochrysisgalabana* | *Algae* | SD2 |
| 4. | *Spirulina platensis* | *Algae* | TA1 |
| 5. | *Sargassumwightii* | *Algae* | SS1 |
| 6. | *Octopus membranaceus* | Molluscan | SK5 |
| 7. | *Crassostreamadrasensis* | Molluscan | SK6 |
| 8. | *Sepia pharaonis* | Molluscan | SK7 |
| 9. | *Loligoduvauceli* | Molluscan | SK8 |
| 10. | *Chlorella salina* | Molluscan | PV1 |


**Fig. 1 .** Microscopic images of selected microalgae with their cell diameter (A) *C. salina,* (B) *C. calcitrans* (C) *T. tetrahele,*(D) *S. platensis,* (E) *I. galbana*

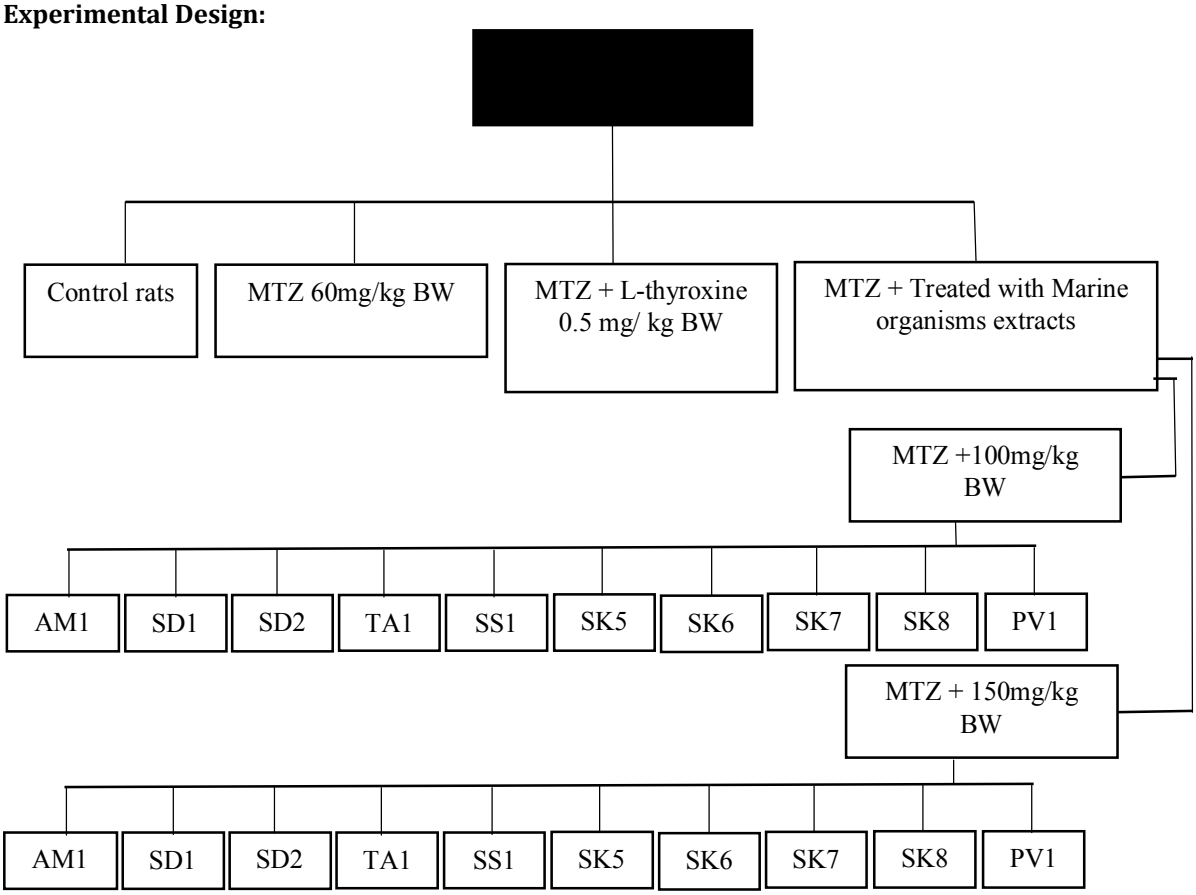
**Maintenance of animals:**

The study was carried out on male albino wister rats (*Rattus norvingicus*) weighing 190±10g, which were housed in cages separately. The animals were purchased at Sri Venkateswara Enterprises Pvt. Ltd. (Bangalore, Karnataka, India) and maintained at room temperature of 25±2ºC, the humidity of 45-55 % with 12/14 hours' light-dark cycle. The animals were kept in polypropylene cages of size 410x280x150 mm lined with rice husk and fed with a standard pellet diet and water *ad libitum***.** The study was approved by the Indian Animal Ethical Committee (IAEC), Santhiram College of Pharmacy, Nandyal, India (Vide No. 15191PO/RE/5/11/CPCSEA/SCRCP/19/04, dt:23.08.2018.

**Induction of hypothyroidism treatment:**

After the acclimatization process of the rats to the laboratory conditions, Hypothyroidism was induced by oral feeding of 60 mg/kg BW of Methimazole in normal drinking water for 14 days[19]. and the control rats were treated with a normal diet, positive control animals were treated with L-Thyroxine (0.5mg/kg/d)[20]. the hypothyroid-induced rats were treated with marine organism extracts by oral administration, dosage as follows 100mg/kg and 150mg/kg body weight.

**Experimental Design:**




**Sample collection for analysis:**

The blood samples were collected from the retro-orbital vein and used for the estimation of serum for hypothyroid. T3, T4, and TSH estimations were done for hypothyroid with VIDAS kits by using VIDAS-PC (BIOMERIX, Model No. IVD7001738, Bangalore) with marine organism extracts (n=10).

**Isolation of tissues:**

After treatment, rats were euthanized and sacrificed humanely by cervical dislocation, and liver, kidney, brain, and thyroid tissues were collected and rinsed of any adhering blood. The tissue fragments were homogenated in phosphate buffer saline for further studies. The unused tissues were stored at –40oC refrigerator for further analysis. The tissues for histopathology were stored in 10 % paraformaldehyde until tissue sectioning.

**Biochemical analysis**

The superoxide dismutase[21], catalase[22], glutathione peroxidase[23], glutathione *S*-transferase[24], reduced glutathione[25], lipid peroxidation[26], activity levels were estimated in the thyroid gland, liver, kidney, and brain tissues of MTZ induced hypothyroidism and treated rats**.**

**T3, T4, and TSH Estimations**

The blood samples were collected from the retro-orbital vein and used for the estimation of T3, T4, and TSH by using VIDAS-PC (BIOMERIX, Model No. IVD7001738, Bangalore).

**Histopathology**

For histological examination, tissues like the thyroid gland, liver, kidney, and brain were excised by euthanizing the rats after treatment. The tissues were fixed in 10% formaldehyde and dehydrated in 50%–100% ethanol, cleared in xylene and paraffin embedding. The sections (10 µm) were stained with hematoxylin and eosin (H & E) dye and examined under a microscope.

**Statistical analysis**

The estimations data were analyzed with the statistical package of social sciences (SPSS, 16.0 version). A comparison between the control and experimental animals results wasdone by one-way ANOVA followed by Tukeys multiple comparison test. $P < 0.05$ was considered as significant difference. All the values were expressed as mean ± SD (n = 6).

## RESULTS

In the present investigation, a total of ten different marine organisms were selected for the screening of the anti-hypothyroid properties in Methimazole-induced hypothyroid rats (Fig.1). In earlier reports, some of the marine organisms have shown significant recovery at 100,150, 200, and 250 mg/kg BW in methimazole-induced hypothyroid rats. Hence 100 and 150 mg/Kg/BW concentrations for hypothyroid of marine organisms were selected to treat the MTZ-induced hypothyroidism rats in our studies. The present study demonstrated that all the marine organism extracts have recovered the altered T3, T4, and TSH levels in methimazole-induced hypothyroid rats. (Fig.2). Among the 10 marine organism extracts TA1 extract at 150 mg/kg BW concentration has shown significant ($P$ <0.05) recovery in altered T3-triiodothyronine (0.66 ± 0.17; 0.86± 0.11) (Fig. 2A) T4 – thyroxine (8.5±0.71; 9.02 ± 0.91)(Fig. 2B) TSH – thyroid-stimulating hormone (1.18±0.05;0.98±0.09)(Fig. 2C) levels compared to diseased (0.19±0.1;2.13±0.21;3.02±0.06) groups.

Among 10 marine macroalgae extracts, TA1 was selected. The TA1 compound was selected mainly based on the T3, T4, and TSH levels (Fig.2). The perturbations of the *in vivo* antioxidant enzyme levels were studied by using the selected potential extract during methimazole-induced hypothyroid rats.

After inducing the thyroid, the levels in thyroid, liver, kidney, and brain antioxidant contents were significantly (P<0.05) altered in MTZ induced hypothyroism rats compared to normal rats. The enzymatic antioxidant such as SOD (4.89±0.76; 3.77±0725; 92±0.75; 0.96±0.15), (Fig.3A) CAT (0.17±0.05; 0.11±0.01; 0.12±0.01; 0.10±0.09), (Fig.3B) GPx (16.95±0.76; 12.70±0.51; 22.36±0.89; 11.42±0.81), (Fig.3C) GST (12.99±0.73; 8.29±0.98; 14.51±0.91; 4.94±0.63), (Fig.4A) GSH (10.54±0.63; 17.21±0.68; 25.73±0.93; 10.54±0.70). (Fig.4B) were significantly ($P$<0.01) decreased, at the same time as the levels of malondialdehyde (MDA) increased (35.64±0.93; 28.38±0.72; 40.35±0.80; 24.62±0.46) (Fig.4C) activity in MTZ induced hypothyroid, rats. The TA1 (150 mg/kg body weight/day) recovered the altered enzyme activities significantly in thyroid rat tissues. TA1 has showed greater ability in reducing the altered levels of antioxidant enzymes activities such as (Fig. 3) SOD (9.48±0.61; 7.49±075; 8.24±0.82; 3.13±0.54), (Fig. 3)CAT (0.47±0.03; 0.40±0.02; 0.43±0.05; 0.35±006), (Fig. 3) GPx (32.7±0.55; 26.23±0.76; 32.60±0.89; 18.51±0.95). (Fig.4) GST (17.25±0.89; 15.59±0.86; 17.52±0.77; 10.84±0.80). (Fig.4) GSH (26.06±0.84; 28.30±0.93; 33.53±0.49; 17.57±0.77). and (Fig.4) LPx (26.06±0.84; 21.92±0.93; 33.53±0.49; 17.57±0.77) levels were recovered The TA1 treated rats does not have any significant (P < 0.05) changes compared to healthy control group.

Histopathological changes in brain, liver and thyroid tissues of MTZ induced hypothyroid TA1 treated (150 mg/kg body weight) rats are shown in (Fig.5). In the brain moderate glial shrubberies, necrosis of multifocal neurons, and deterioration of cells were observed in hypothyroidism rats (Fig. 5A). In thyroid rat kidney, uneven thickening of the basement membrane, endolysins, local fusion of the foot process of the podocytes, and glomerular capillary endothelium swelling was observed (Fig. 5D). The disordered structure of the liver in hypothyroidism rats was observed because of necrosis of hepatocytes, extensive vacuolization with the disappearance of nuclei, and microcellular fatty changes (Fig.5G). . thyroid gland of (J) control, (K) MTZ- Induced (follicular cells and parafollicular cells) (L) TA1- treated cells shows cells recovery observed in thyroid rat tissues (Fig. 5J). However, these changes were recovered with *S. platensis* derived TA1 (Fig. 5C, 5F, 5I, 5L).

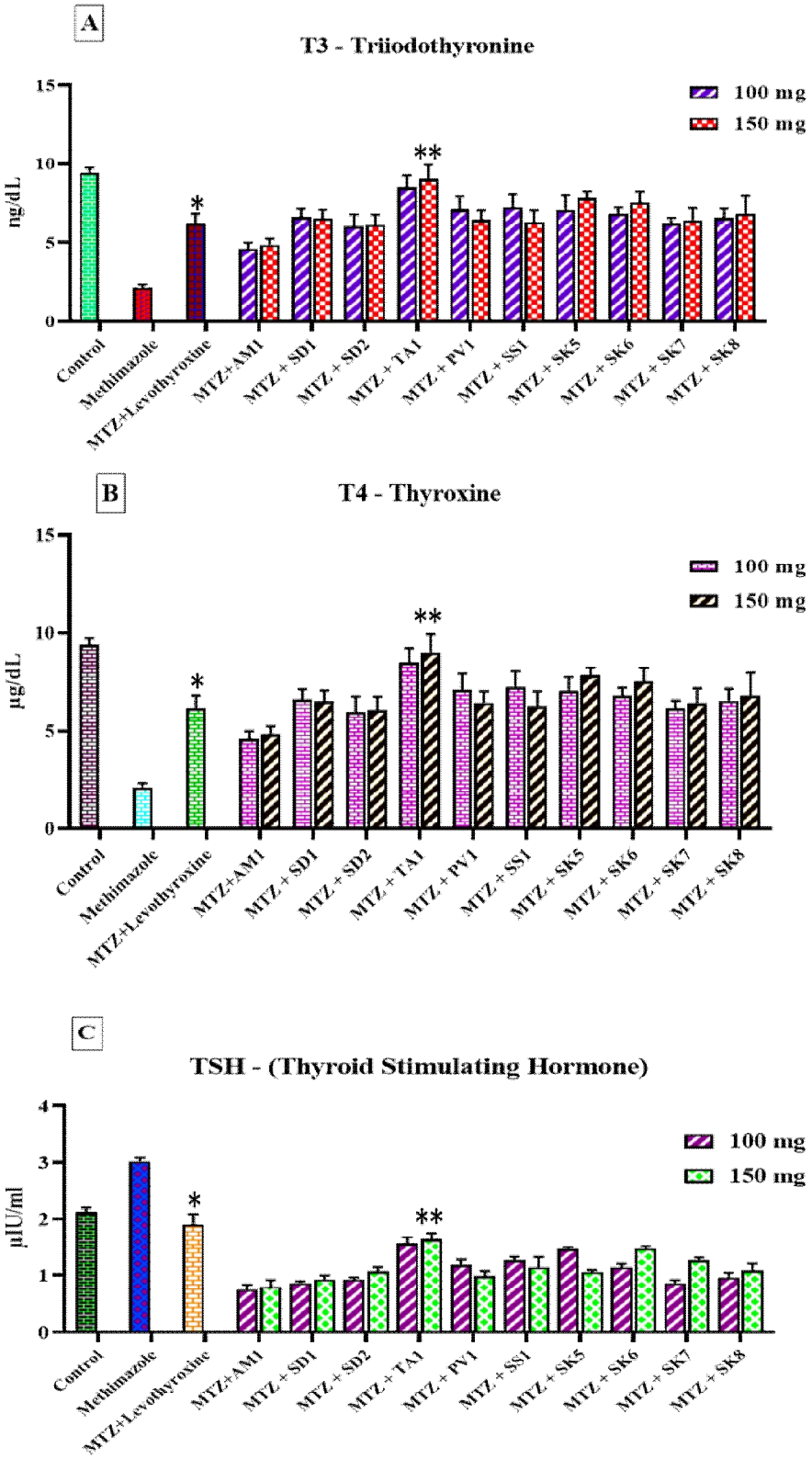



**Fig. 2:**Estimation of serum T3, T4, and TSH levels in methimazole (MTZ) induced hypothyroid rats treated with marine organisms.

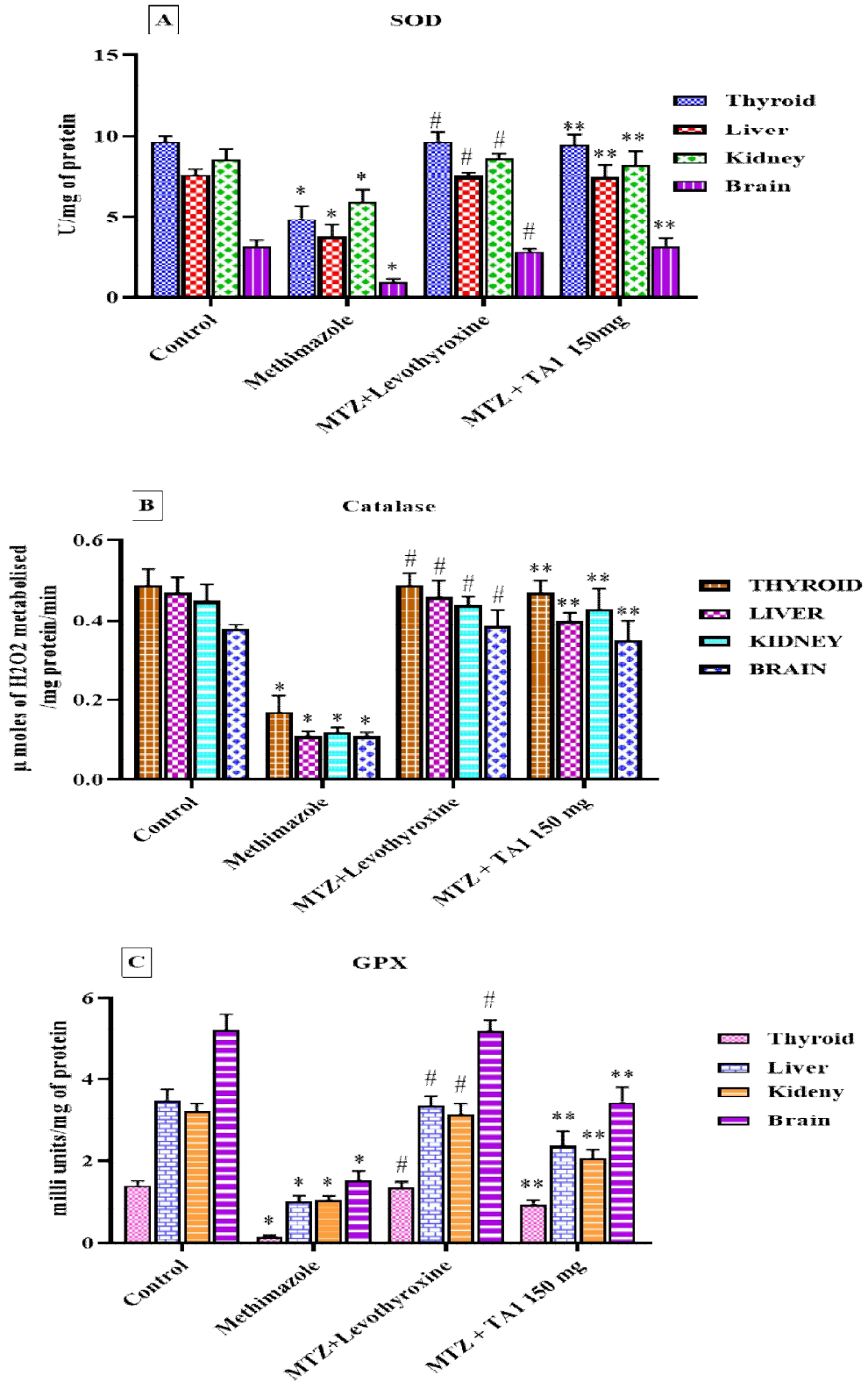



**Fig.3:** Estimation of (A) Superoxide dismutase(SOD), (B) Catalase, (C) Glutathione peroxidase(GPX)levels in Methimazole(MTZ) induced hypothyroidism rats treated with TA1 150mg/kg BW

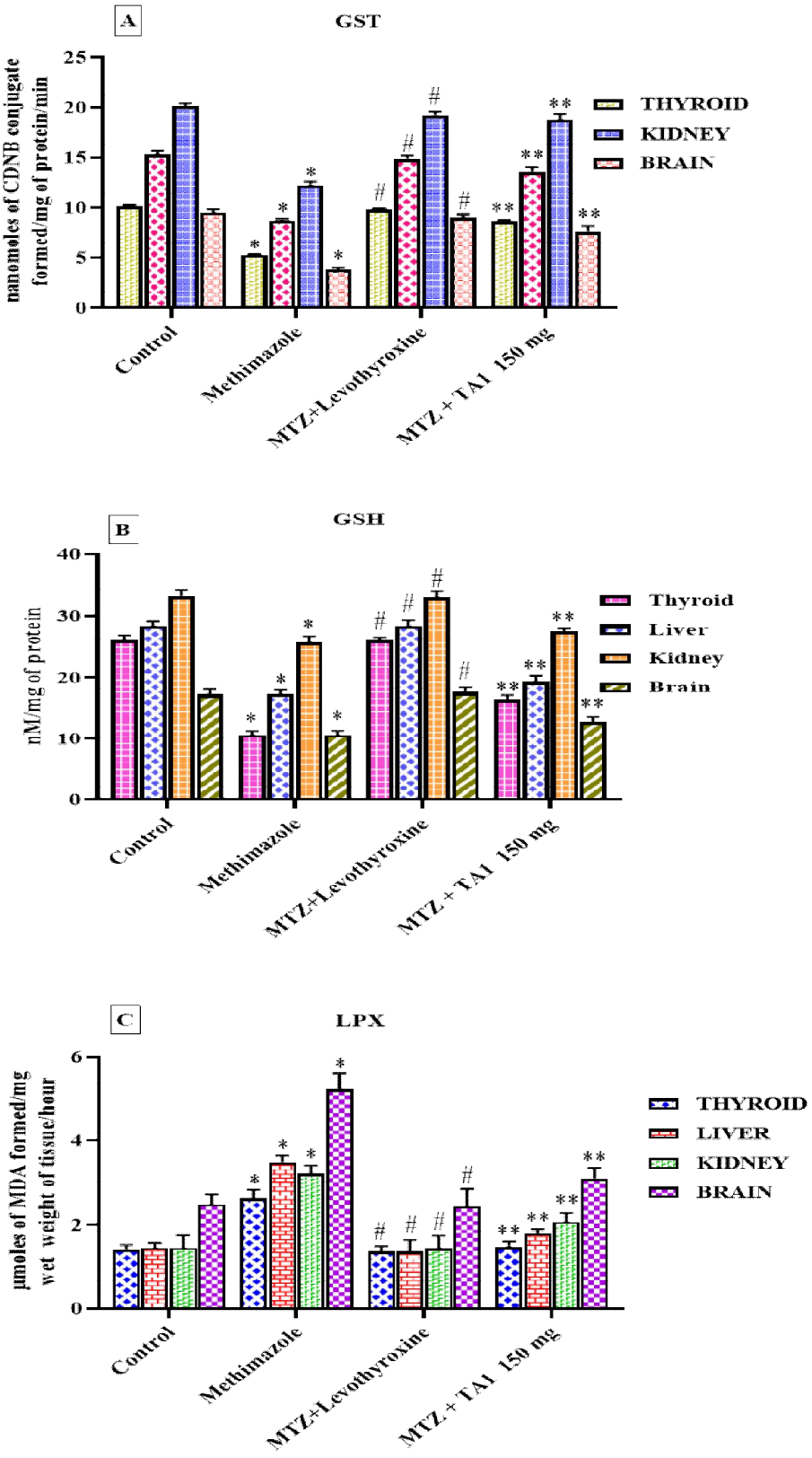



**Fig.4:** Estimation of (A) Glutathione S-Transferase (GST), (B) Reduced glutathione(GSH), (C) Lipid Peroxidation (LPX) levelsin Methimazole (MTZ) induced hypothyroidism rats treated with TA1 150mg/kg BW

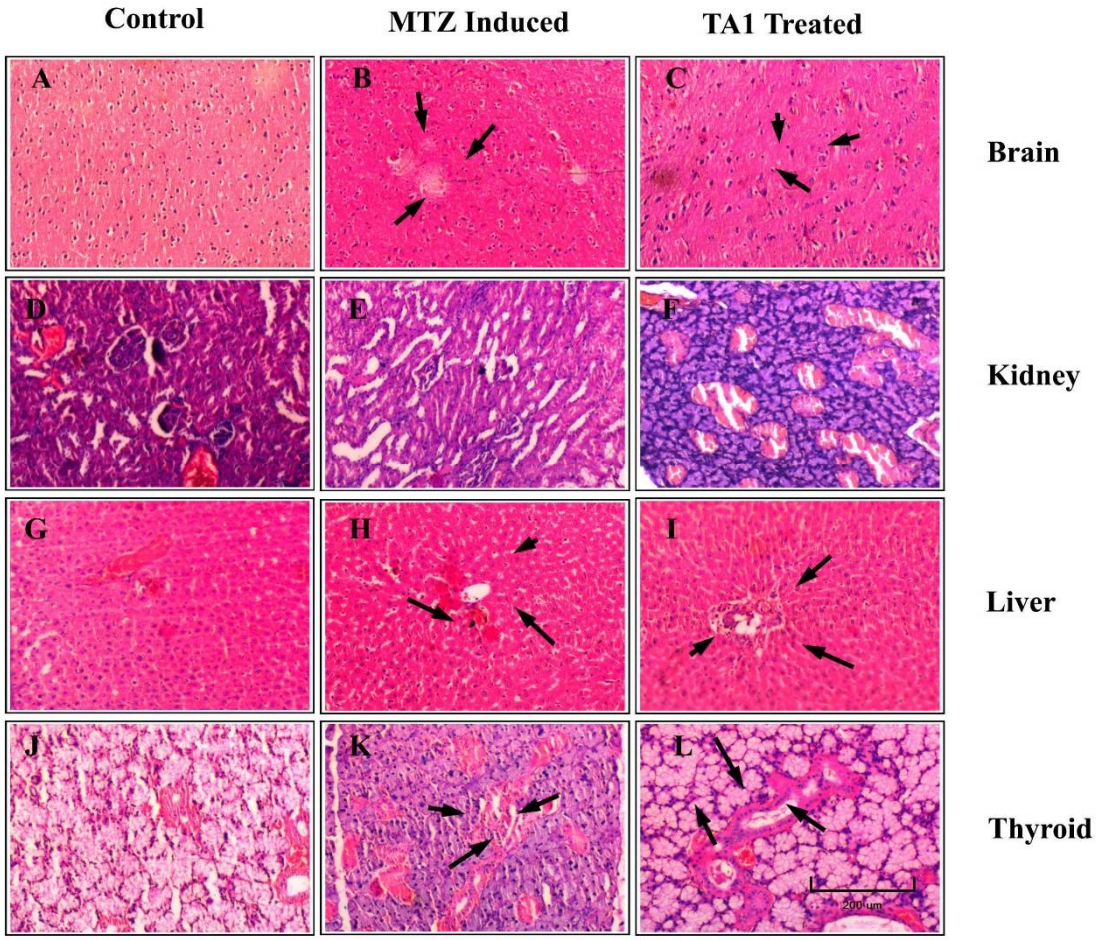



**Fig. 5:** Photomicrographs of A) Thyroid gland, Liver, Kidney, and Brain of MTZ induced hypothyroidism rats.

**DISCUSSION**

*Spirulina platensis* (TA1) has been used in folk medicine as an anti-inflammatory and anti-oxidative agent throughout the world[27]. It has been believed that hypothyroid leads to oxidative and T3, T4, TSH damage of organs, and anti-oxidants have been reliable and favorable effects on hypothyroid. It is also expected that TA1 extracts also may be shown beneficial for hypothyroidism and related organ damages because TA1-*Spirulina platensis* extracts have been showing various pharmacological and anti-oxidant effects. In the present study, we investigated the effects of TA1 aqueous extracts on methimazole-induced hypothyroidism and organ damages in comparison with Levothyroxine treated rats with their possible anti-oxidant and T3, T4, TSH levels. Among the 10 extracts, TA1 has shown significant alterations in serum biomarkers compared to the diseased group(Fig.2). This was previously reported in*Bauhinia variegata* and *Commiphora mukul*[28].

It has been well documented that thyroid dysfunctions increase LPx reactions and reactive oxygen species (ROS). LPx is an autocatalytic mechanism leading to oxidative destruction of cellular membranes. Such destruction can lead to cell death and to the production of toxic and reactive aldehyde metabolites called free radicals, where malondialdehyde(MDA) is the most important. It is known that ROS would lead to oxidative damage of biological macromolecules, including lipids, proteins, and oxidative stress also influenced to the body adipocyte results in a decrease of body fat masses and related body weight decreases. MDA is a terminal product of LPx. Hence the content of MDA can be used to estimate the extent of LPx, and marked increases of the thyroid gland, liver, kidney, brain MDA contents have been observed in hypothyroid animals. GSH is a representative endogenous antioxidant, prevents tissue damage by keeping the ROS at low levels and certain cellular concentrations, and is accepted as a protective antioxidant factor in tissues. SOD is one of the antioxidant enzymes that contribute to enzymatic defense mechanisms, and catalase is an enzyme that catalyzes the conversion of $H_2O_2$ to $H_2O$. The increase of some antioxidant enzyme activities such as SOD and catalase may be indicative of the failure of compensating

the induced oxidative stress. In hypothyroidism, it is well known that marked decreases of tissue GSH contents were induced, represent the decrease of antioxidant defense systems. Controversially, SOD, Catalase. GPx and GST) activities increased to remove over-produced ROS as of indication of the failure of compensating the induced oxidative stress. Methimazole-induced hypothyroidisms oxidative stresses and related organ damages were ameliorated by treatment of TA1 extracts in the present study like other previously tested antioxidants, as direct evidence that TA1 extracts have potent antioxidant effects enough to inhibit hypothyroidism. However, further mechanism studies should be conducted to clarify whether TA1 reduced oxidative damages of vital organs via improvement of thyroid function because dysfunction of thyroid hormones also can be lead to oxidative damages. The antioxidant activities of different tissues were previously reported in *Sargassum hemiphyllum* [29]

Histopathological changes were observed in Methimazole induced hypothyroidism rats tissue of thyroid gland, brain, liver, and kidney. The histopathological change like sub-glial cells and neurons in the brain, intense vascular degeneration of hepatocytes, and in the parenchyma in the liver, and follicular cells, and parafollicular cells in the thyroid gland were the prominent histopathological alterations observed in the Methimazole induced Hypothyroidism rats for 28 days. These alterations were recovered to a normal state with the treatment of TA1 (Fig.5). These studies provide evidence that the increased levels of free radicals generated are the causes of tissue damage[30], which is clear by the high amount of lipid peroxidation that is accompanied by an improper antioxidant enzyme state.[31]stated the reasons for tissue damage are due to an increase in the production of free radicals. In this current study, it was observed high levels of lipid peroxidation were accumulated which was then accompanied by a decrease in the antioxidant enzyme are due to the free radicals generated and might be the leading causes for architectural damage in tissues. It was previously reported that sulfated polysaccharides from other marine macroalgae were considered as safe on toxicological evaluation.[32]

## CONCLUSION

In the present investigation, the effect of marine organism's extract of *Spirulina platensis* (TA1) on serum T3, T4, and TSH levels was evaluated in MTZ induced hypothyroidism rats. Animal studies have shown that, compared to other treatment groups, a combination of high dose (150mg /kg body weight) of *Spirulina platensis* (TA1) showed a significant effect on serum T3, T4, and TSH levels. The study showed that the combined extract dose of *Spirulina platensis* significantly reduced the serum T3, T4, and TSH levels through a synergistic effect. This might be a potential therapeutic alternative for the management of hypothyroidism. However, further investigation is required to establish these marine organisms extract as a safe and effective natural product.

## ACKNOWLEDGMENT

This work was supported by the Ministry of Science and Technology of Department. of Biotechnology(DBT), Govt of India, Delhi (grant number BT/PR15757/ AAQ/ 31757/2015).

## CONFLICT OF INTEREST

No conflict of interest was reported by the authors.

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## CITATION OF THIS ARTICLE

Navyashree B, Anisha S Jain, Sushma P, Chandan D, Sharagouda S Patil, Shiva Prasad Kollur, Latha KC and Chandan S. A Computational based approach in discovering the phytocompound based complex inhibition against *Ralstonia solanacearum* and root-knot nematode *Meloidogyne incognita*. Bull. Env. Pharmacol. Life Sci., Vol 10[11] October 2021 : 42-52.