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Therapeutic Evaluation of Selected Herbal Supplements on Thyroid Hormones of Cyanide – Induced Hyperthyroidism in Female Albino Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: To evaluate the therapeutic effect of some herbal supplements on thyroid hormones of cyanide – induced hyperthyroidism in Female Albino Rats.
Study Design: Experimental study.
Place and Duration of Study: Department of Animal and Environmental Biology, Rivers State University, Rivers State University Teaching Hospital and Department of Pharmacology, University of Port Harcourt, Nigeria, between July and September, 2020.
Methodology: 150 female albino rats were used for this study. The rats were divided into ten groups of fifteen rats each: group A-negative control, group B-positive control, group C- orthodox drug (propranolol), group D-herbal supplement (motherwort), group E-bugleweed, group F-G*arcinia kola*, group G-propranolol and bugleweed, group H-propranolol and motherwort, group I-propranolol and G*arcinia kola*, and group J-bugleweed and motherwort. Hyperthyroidism was induced in groups B to J by the oral administration of 2.4 mg/kg of potassium hexacyanoferrate III salt and given every two days to sustain the induction. The rats were treated with the drug, supplements and seed extract for 14, 30 and 60 days. On the 15th, 31st, and 61th days after overnight fast, the rats were anesthetized with chloroform and sacrificed through cardiac puncture. 5ml of blood samples was put into plain bottles for the analysis of thyroid hormones. The thyroid

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function (triiodothyronine -T_3, thyroxine -T_4, and thyroid stimulating hormone -TSH) were analyzed using the ELISA technique. GraphPad Prism 5.6. was used to analyze the data and mean values were considered statistically significant at *P< .05.*

Results: The results showed that there were significant increases (*p<.01*) in the levels of T3 and T4 and decreases in TSH levels for days 14, 30 and 60 of the experiment after rats were exposed to cyanide. Treatment with the herbal products at some points significantly reduced T3 andT4 levels, while TSH levels were significantly increased. The combination therapies used in this study did not offer significantly different therapeutic advantage over the individual therapies.

Conclusion: Cyanide exposure in rats caused hyperthyroidism, but administration of some herbal supplements ameliorated the effect of cyanide, therefore, more studies on these supplements are suggested.

Keywords: Herbal supplements; thyroid hormones; cyanide; hyperthyroidism; female albino rats.

1. INTRODUCTION

Hyperthyroidism is a condition that arises when the thyroid gland produces too many thyroid hormones. It can cause unexpected weight loss and a quick or irregular pulse by speeding up the body's metabolism [1,2]. Thyroid hormones are secreted by the thyroid gland, which is one of the largest endocrine glands in the body [3,4]. Thyroid disease is a medical illness that affects the thyroid gland's ability to operate. Thyroid diseases are more common in women than in men, with a 4:1 prevalence ratio for thyroid illness. There are five general types of thyroid disease, each with its own symptoms. It is possible to have one or several different types at the same time. The different types of the disease are hypothyroidism, a low function which is caused by not having enough free thyroid hormones; hyperthyroidism, a high function having too much of the thyroid hormones; on data of community-based studies the prevalence of hyperthyroidism in female is 2% and in male 0.2%, and about 15% of patient of hyperthyroidism occurring in old age patient above 60 years of age. Structural abnormalities which is the enlargement of the thyroid gland most commonly a goiter; tumors which can be benign or cancerous; and abnormal thyroid function tests without any clinical symptoms that can be subclinical hypothyroidism or subclinical hyperthyroidism [5,6]. In some types, such as sub-acute thyroiditis or postpartum thyroiditis, symptoms may go away after a few months and laboratory tests may return to normal [7]. However, most types of thyroid disease do not resolve on their own due to environmental factors.

Cyanide is one of the major environmental pollutants and is termed a thyroid disruptor [8,9]. Regardless of its origin, it is a primary toxic agent [10]. Large proportion of the population are exposed to very low levels of cyanide in the general environment. When exposed to this hazardous chemical, several factors can determine whether harmful health effect will occur and what type and severity of the health effects will be. These factors include the dose (how much), the duration (how long), the route or pathway by which one is exposed (breathing, eating, drinking, or skin contact), the other chemicals to which it is exposed and the individual characteristics such as age, sex, nutritional status, family traits, life style and state of health. Environmental factors of cyanide have been associated with much intoxication in humans and animals resulting from exposure to environmental pollution, chemical war and industrial sources, which are forms of occupational hazard [11,12], can lead to structural abnormalities and may not produce symptoms; however, some people may have hyperthyroid or hypothyroid symptoms related to the structural abnormalities or notice swelling of the neck [13]. Cyanide poisoning is mostly due to the stoppage of aerobic cell metabolism. Cyanide attaches to the ferric ion cytochrome oxidase three within the mitochondria in a reversible manner. This effectively stops cellular respiration by preventing oxygen from being reduced to water [14]. The principal effect of cyanide is that it inhibits oxidative phosphorylation, a process in which oxygen is used to produce ATP, a vital cellular energy source. It accomplishes so by blocking the mitochondrial transport chain by attaching to the enzyme cytochrome C oxidase. Cellular hypoxia and ATP depletion follow, resulting in metabolic acidosis. The use of oxygen by tissue happens, and this is followed by a loss of important activities.

Treatment of hyperthyroidism varies and based on the disorder. Its clinical characteristics are

complex, internationally conventional drugs such as propranolol/ metoprolol are most frequently used to augment treatment for hyperthyroidism, because propranolol is a beta blocker without intrinsic sympathomimetic activity which are effective therapeutic adjuncts in the management of hyperthyroidism [15]. Medicinal herbs, on the other hand, have been recognised and used throughout human history. The effects of plants on the human body are mediated through chemical compounds found in plants. Herbal medication has fewer adverse effects, and the herbs and spices that humans use to season their meals also contain medicinal ingredients. In recent years, medicinal plants, particularly those employed in the treatment of hyperthyroidism, have attracted a lot of attention. Plant-derived drugs are thought to be more safer, have a high effectiveness, and play an important role in human-environmental interactions [16].

There are claims that herbal supplements are better therapies for hyperthyroidism mainly due to the complex etiology of the disease [16]. Currently, the drugs used for the treatment of this disease have been reported to have adverse side effects [17], and so, the herbal supplementations are suggested as a viable substitute to drugs presently used in the management of hyperthyroidism. Chemical compounds of orthodox drugs such as propranolol mediates effect on the human body. Herbal supplements such as bugleweed and motherwort produce lesser side effects. Bugleweed is a plant drug which is used in the management of thyroid disorder and which have a direct action towards alleviating hyperthyroidism. Bugleweed is effective in blocking the binding of TSH to the receptor by acting on the hormone and the receptor itself. It also inhibits cyclic AMP production stimulated by TSH receptor antibodies. Motherwort is used in the management of autoimmune diseases which is important in the reduction of inflammation, making motherwort a good choice in the treatment of hyperthyroidism. In addition to reducing inflammation, the enzyme 5 – deiodanase is inhibited. It is an herbaceous perennial plant in the mint family of Lamiaceae. The parts that grow above the ground are used to make medicine. *Garcinia kola* is largely cultivated forest tree indigenous to sub – Saharan Africa. It has been described as a wonder plant because of almost every part of this wonder plant has been found to be of medicinal importance. The seed is masticatory used in traditional hospitality, cultural and social ceremonies. Extracts of the plant have been used traditionally for aliments such as liver diseases, cold, cough and has anti – inflammatory, antimicrobial, anti-diabetic and antiviral as well as antiulcer properties. The aim of this study was to evaluate the therapeutic effect of some herbal supplements on thyroid hormones of cyanide – induced hyperthyroidism in Female Albino Rats.

2. MATERIALS AND METHODS

2.1 Experimental Animals

One hundred and fifty (150) female albino rats weighing between 150 – 200g were obtained from the Pharmacology Department, University of Port Harcourt, Nigeria, and kept in well aerated laboratory cages in the Animal House, Department of Biological Sciences, Rivers State University, Port Harcourt, Rivers State, Nigeria. The animals were allowed to acclimatize to the laboratory environment for a period of fourteen days (14 days) before commencement of the experiment. All animals were fed with standard commercial rat feed and water *ad libitum*.

2.2 Purchase of Propranolol, Bugleweed, Motherwort and *Garcinia Kola* Seeds

The orthodox drug used for the study was Propranolol (Propranolol Hydrochloride) a product of Scott – Edil Pharmacia, India. The supplements used were Bugleweed (*Lycopus virginicus*) and Motherwort (*Leonurus cardiac*), products of Swanson Health products, USA, as well as *Garcinia kola* (Bitter kola) seed. The orthodox drugs were purchased in Ebus Pharmaceutical Shop Port Harcourt and supplements were purchased from Amazon's shop USA, while the *Garcinia kola* seeds were purchased from a reputable dealer at mile 3 markets in Port Harcourt city.

2.3 Preparation of Extract of *Garcinia Kola* Seed

The seeds of *Garcinia kola* were washed, de-husked and cut into small pieces. They were then dried in hot air oven at 45^{o}C for 24 hours and allowed to cool. *Garcinia kola* seeds (400 g) cut into pieces was weighed and soaked in 96% of ethanol in a volumetric flask. The extraction was carried out in a Soxhlet extractor at 62^{o}C for 72 hours. The extract was evaporated to dryness in vacuum at 40^{o}C and a constant yield following

repeated weighing was found to be 383 g indicating the complete removal of ethanol from the extract. The extract was stored in a refrigerator at – 65^{o}C until used for the experiment. The extract was reconstituted in distilled water for the oral administration to the animals designated for the experiment as described by Olutayo et al. [18].

2.4 Determination of Therapeutic Dose

The rat doses of the herbal formulations and orthodox drug were extrapolated from the human therapeutic doses based on body surface area ratio using the conversion table which is based on 70kg as the weight of adult human and 200 g as the rat weight.

Rat dose for each drug was calculated using the formula:

Rat Dose (mg/kg) = Human Dose (mg) x 0.018 x 5.

The daily dose of both the orthodox drug and the herbal supplements were determined based on the Organization for Economic Co-operation and Development's [19]. The drug and supplements were dissolved in sterile water and administered to the rats accordingly.

2.4.1 Calculation of Doses

2.4.1.1 Motherwort (Leonurus cardiaca)

Each capsule is 400mg which is the dosage for adult human (70kg) taken once daily making it 400 mg/day.

Rats Dose (mg/kg) = Human Dose x 0.018 x 5

400 mg x 0.018 x 5 = 36 mg/kg

Therefore, daily dose for rat (200 g) = weight of rat/1000 x standard dose

200/1000 x 36 mg = 7.2 mg

According to OECD [19] guideline, this dosage should be dissolved in 2 ml of distilled water.

Thus, if 7.2 mg of Motherwort was to be dissolved in 2 ml of water then 400 mg (one capsule) will be dissolved in 2 x 400/7.2 = 111 ml of diluent.

To prepare the stock, one capsule of Motherwort was dissolved in 111 ml of distilled water. This was done weekly.

2.4.1.2 Bugleweed (Lycopus virginicus)

Each capsule contains 400 mg. Dosage for adult human is one capsule taken twice daily making it 800 mg.

Rat Dose (mg/kg) = Human dose x 0.018 x 5 = 800 x 0.018 x 5 = 72 mg/kg

Daily dose for rat using 200 g = weight of rat x standard dose/1000 = 200x72/1000 = 14.4 mg

According to OECD [19] guidelines, this dosage is to be dissolved in 2 ml of distilled water.

Thus, if 14.4 mg of Bugleweed should be dissolved in 2 ml of water then 400 mg (one capsule) will be dissolved in 2 x 400/14.4 = 55.5 ml of diluent.

To prepare the stock, one capsule of Bugleweed was dissolved in 55.5 ml of distilled water. This was done weekly.

2.4.1.3 Propranolol Hydrochloride

Each tablet contains 40 mg. Dosage for human (70 kg) is one tablet taken three times daily giving it 120 mg/day.

Rat Dose (mg/kg) = Human dose x 0.018 x 5 = 120 x 0.018 x 5 = 10.8 mg/kg

Daily rat dose (200 g) = weight of rat/1000 x standard dose = 200/1000 x10.8 = 2.16 mg

According to OECD [19] guidelines, this dosage should be dissolved in 2 ml of distilled water. Thus, if 2.16 mg of propranolol is to be dissolved in 2 ml of distilled water, then 40 mg will be dissolved in 2 x 40/2.16 = 37 ml of diluent.

2.4.1.4 Garcinia kola (Bitter cola)

There was no mortality in this LD_{50}, so the dose to be used will be 5 ml (5000 mg/kg).

Rat dose (mg/kg) = Human dose x 0.018 x 5 = 5000 x 0.018 x 5 = 450 mg/kg.

Daily rat dose = of weight 200 g = weight of rat/1000 x standard dose = 200/1000 x 450 = 90 mg

According to OECD [19] guidelines, this dosage should be dissolved in 2 ml of distilled water. Thus, if 90 mg of *Garcinia Kola* is to be dissolved

in 2 ml of water then 5000 mg will be dissolved in 2 x 0.5/0.09 = 11.1 ml of diluent.

2.5 Induction of Hyperthyroidism and Treatment with Herbs

From a previously conducted pilot toxicity study, 2.4 mg/kg was used to induce hyperthyroidism in rats, Adeniyi et al. [20]. Hyperthyroidism was induced in the rats, after which the rats were treated with the herbal supplements (Bugleweed and Motherwort), *Garcinia kola* and orthodox drug (Propranolol) which lasted for 14 days, 30 and 60 days. This treatment was carried out at 8:00 am, given through oral gavage once daily before the animals were fed for the period of the fourteen, thirty and sixty days. The drug and supplements were given in soluble form (aqueous) while the *Garcinia kola* was given as an extract.

2.6 Experimental Design

One hundred and fifty (150) female albino rats were divided into ten (10) groups of fifteen (15) rats each in a cage as follows:

(a). Group A: Hyperthyroidism was not induced in this group and serves as negative control.
(b). Group B: Hyperthyroidism was induced using 2.4 mg/kg of $K_3Fe(CN)_6$ and served as a positive control.
(c). Group C: Hyperthyroidism was induced using 2.4 mg/kg of $K_3Fe(CN)_6$ and treated with 2.16 mg/kg of propranolol hydrochloride for 14, 30 and 60 days.
(d). Group D: Hyperthyroidism was induced using 2.4 mg/kg of $K_3Fe(CN)_6$ and treated with 7.2 mg/kg of motherwort for 14, 30 and 60 days.
(e). Group E: Hyperthyroidism was induced using 2.4 mg/kg of $K_3Fe(CN)_6$ and treated with 14.4 mg/kg of bugleweed for 14, 30 and 60 days.
(f). Group F: Hyperthyroidism was induced using 2.4 mg/kg of $K_3Fe(CN)_6$ and treated with 90 mg/kg of garcinia kola for 14, 30 and 60 days.
(g). Group G: Hyperthyroidism was induced using 2.4 mg/kg of $K_3Fe(CN)_6$ and treated with a combination therapy of propranolol hydrochloride and bugleweed for 14,30 and 60 days.
(h). Group H: Hyperthyroidism was induced using 2.4 mg/kg/kg of $K_3Fe(CN)_6$ and treated with a combination therapy of propranolol hydrochloride and motherwort for 14, 30 and 60 days.
(i). Group I: Hyperthyroidism was induced using 2.4 mg/kg of $K_3Fe(CN)_6$ and treated with a combination of propranolol and garcinia kola for 14, 30 and 60 days.
(j). Group J: Hyperthyroidism was induced using 2.4 mg/kg of $K_3Fe(C N)_6$ and treated with a combinations of motherwort and bugleweed for 14, 30 and 60 days

2.7 Collection of Samples

2.7.1 Blood sample

Twenty-four (24) hours after last administration, the animals were sacrificed after an overnight fast on the fifteenth, thirty first and sixty first days. They were anaesthetized using chloroform in a desiccator to ameliorate suffering and cardiac puncture was performed, 5 ml of whole blood were collected into plain bottles, centrifuged at 3000 rpm for 5 minutes to obtain serum for biochemical analysis.

2.8 Laboratory Analysis

2.8.1 Estimation of triiodothyronine using rat ELISA technique

2.8.1.1 Principle

The microtiter wells were coated with Triiodothyronine (T_3) EIA, a second antibody and a measured amount of rat serum, monoclonal anti–triiodothyronine (T_3) antibody, Triidothyronine (T_3) conjugated with horseradish peroxidase are also added into the microtiter wells. During incubation, the anti- T3 antibody is bound to a second antibody on the wells, and T_3 and conjugated T_3 compete for the limited binding sites on the anti-T_3 antibody. After incubation, unbound T_3 conjugate are washed away and a solution of tetramethylbenzidine (TMB) reagent was added and incubated for some minutes resulting in the development of blue color. The color development is stopped with the addition of stop solution, and the absorbance is measured spectrophotometrically at 450nm.

2.8.2 Estimation of thyroxine using Rat ELISA technique

2.8.2.1 Principle

The microtiter wells were coated with T_4 EIA, a second antibody and a measured amount of rat

serum, monoclonal anti –T_4 antibody, T_4 conjugated with horseradish peroxidase are also added into the microtiter wells. During incubation, the anti- T_4 antibody is bound to a second antibody on the wells, and T_4 and conjugated T_4 compete for the limited binding sites on the anti-T_4 antibody. After incubation, unbound T_4 conjugate are washed away and a solution of tetramethylbenzidine (TMB) reagent was added and incubated for some minutes resulting in the development of blue color. The color development is stopped with the addition of stop solution, and the absorbance is measured spectrophotometric ally at 450 nm.

2.8.3 Estimation of thyroid stimulating hormone using Rat ELISA technique

2.8.3.1 Principle

The TSH ELISA test is based on the principle of solid phase EIA which utilizes a unique monoclonal antibody that is directed against a distinct antigenic determinant on the intact TSH molecule. The monoclonal anti- TSH antibody is used for a solid phase immobilization on the microtiter wells. The anti- TSH antibody is in the horseradish peroxidase conjugate solution and the test sample is allowed to react simultaneously with the two antibodies resulting in the TSH molecules being sandwiched between the solid phase and enzyme linked antibodies. After incubation at room temperature, the wells are washed to remove unbound labeled antibodies, a solution of TMB reagent is added and incubated for some minutes, resulting in the development of blue color which was stopped by the addition of stop solution changing the color to yellow. The concentration of TSH is directly proportional to the intensity of the test sample. The absorbance is measured at 450 nm spectrophotometrically.

2.9 Statistical Analysis

Values were reported as mean ± standard error of the mean (SEM). Significance was determined statistically by the application of one-way analysis of variance (ANOVA) with a Tukey's multiple comparison test using the statistical software GraphPad Prism 5.6. Differences between means were considered statistically significant at $P< .05$

3. RESULTS AND DISCUSSION

This study investigated the therapeutic effect of three supplements used in the treatment of cyanide-induced hyperthyroidism. The results were used to assess the effect of these agents either individually or in combination against the effect of cyanide in the albino rats.

The parameters used to assess the thyroid damage/injury were triiodothyronine, thyroxine and thyroid stimulating hormones. The thyroid is an organ of importance. The study demonstrated that cyanide causes serious damage to thyroid gland by inducing alteration upon the administration of 2.4mg/kg of it to the rats. Triiodothyronine and thyroxine constitute most standard thyroid function tests and play a central role in the process of diagnosis and treatment of

Table 1. Mean ± SD thyroid hormones levels of cyanide – induced hyperthyroid rats 14 days after treatment with drug, herbal supplements and extract

Groups	T_3 (ng/ml)	T_4 (µg/dl)	TSH (µiu/ml)
A (NC)	0.63 ± 0.06	6.67 ± 1.15	0.43 ± 0.06
B (PC)	2.53 ± 0.40	14.80 ± 0.17	0.10 ± 0.01
C (PROP)	2.87 ± 0.12	14.20 ± 0.35	0.10 ± 0.01
D (MOT)	2.33 ± 0.23	13.93 ± 0.11	0.10 ± 0.01
E (BUG)	2.00 ± 0.01	13.83 ± 0.11	0.16 ± 0.05
F (G.K)	2.43 ± 0.06	13.66 ± 0.60	0.13 ± 0.60
G (P+B)	2.93 ± 0.12	13.36 ± 0.12	0.10 ± 0.01
H (P+M)	1.97 ± 0.06	14.03 ± 0.12	0.20 ± 0.01
I (P+G.K)	2.00 ± 0.01	13.56 ± 0.11	0.10 ± 0.01
J (B+M)	2.02 ± 0.06	13.30 ± 0.17	0.10 ± 0.01
P - Values	<0.0001	<0.0001	<0.0001
F - Values	49.03	101.5	32.74

Study was done replicate. ANOVA, followed by Tukey's multiple comparison test. NC = Negative control, PC =Positive control, PROP and P = Propranolol, MOT and M = Motherwort, BUG and B = Bugleweed, G.K = Garcinia Kola, T_3 = Triiodothyronine, T_4 = Thyroxine, TSH = Thyroid Stimulating Hormone

Table 2a. Summary of Group Comparison of Tukey Multiple Comparison Test; Mean ± SD Thyroid hormones levels for the controls and test groups

Groups	T3 (ng/ml)	T4 (µg/di)	TSH (µlu/ml)
Group A vs Group B	***	***	***
Group A vs Group C	***	***	***
Group A vs Group D	***	***	***
Group A vs Group E	***	***	***
Group A vs Group F	***	***	***
Group A vs Group G	***	***	***
Group A vs Group H	***	***	***
Group A vs Group I	***	***	***
Group A vs Group J	***	***	***
Group B vs Group C	Ns	Ns	ns
Group B vs Group D	Ns	Ns	ns
Group B vs Group E	*	Ns	ns
Group B vs Group F	Ns	Ns	ns
Group B vs Group G	Ns	**	*
Group B vs Group FI	**	Ns	ns
Group B vs Group I	*	*	ns
Group B vs Group J	*	**	ns
Group C vs Group D	*	Ns	ns
Group C vs Group E	***	Ns	ns
Group C vs Group F	Ns	Ns	ns
Group C vs Group G	Ns	Ns	*
Group C vs Group H	***	Ns	ns
Group C vs Group 1	***	Ns	ns
Group C vs Group J	***	Ns	ns
Group D vs Group E	Ns	Ns	ns

Key: A =Negative Control, B = Positive Control, C= Propranolol, D= Motherwort, E= Bugleweed, F= Garcinia kola, G= Combination of Propranolol and Bugleweed, H= Combination of Propranolol and Motherwort, I= Combination of Propranolol and Garcinia kola, J= Combination of Bugleweed and Motherwort.

Table 2b. Summary of Group Comparison of Tukey Multiple Comparison Test; Mean ± SD Thyroid hormones levels for the controls and test groups

Groups	T3 (ng/ml)	T4 (µg/di)	TSH (µlu/ml)
Group D vs Group F	Ns	Ns	ns
Group D vs Group G	Ns	Ns	ns
Group D vs Group H	Ns	Ns	ns
Group D vs Group 1	Ns	Ns	ns
Group D vs Group J	Ns	Ns	ns
Group E vs Group F	Ns	Ns	ns
Group E vs Group G	***	Ns	ns
Group E vs Group H	Ns	Ns	ns
Group E vs Group 1	Ns	Ns	ns
Group E vs Group J	Ns	Ns	ns
Group F vs Group G	*	Ns	*
Group F vs Group H	*	Ns	ns
Group F vs Group 1	Ns	Ns	ns
Group F vs Group J	Ns	Ns	ns
Group G vs Group H	***	Ns	*
Group G vs Group 1	***	Ns	*
Group G vs Group J	***	Ns	*
Group H vs Group 1	Ns	ns	ns
Group H vs Group J	Ns	ns	ns
Group 1 vs Group J	Ns	ns	ns

Key: A =Negative Control, B = Positive Control, C= Propranolol, D= Motherwort, E= Bugleweed, F= Garcinia kola, G= Combination of Propranolol and Bugleweed, H= Combination of Propranolol and Motherwort, I= Combination of Propranolol and Garcinia kola, J= Combination of Bugleweed and Motherwort.

Table 3. Effects of treatment of drug, herbal supplements and extract on thyroid hormones of cyanide – induced hyperthyroid rats after 30 days

Groups	T3 (ng/ml)	T4 (µg/dl)	TSH (µiu/ml)
A (NC)	0.70 ± 0.01	7.10 ± 0.66	0.87 ± 0.12
B (PC)	2.70 ± 0.20	13.63 ± 0.10	0.10 ± 0.01
C (PROP)	1.20 ± 0.20	10.60 ± 0.27	1.07 ± 0.29
D (MOT)	1.00 ± 0.20	12.50 ± 0.70	1.47 ± 0.50
E (BUG)	1.27 ± 0.12	12.27 ± 0.46	1.00 ± 0.01
F (G.K)	1.27 ± 0.31	11.33 ± 1.27	1.67 ± 0.57
G (P+B)	1.16 ± 0.28	11.53 ± 0.64	1.83 ± 0.57
H (P+M)	0.93 ± 0.12	11.76 ± 2.22	1.06 ± 0.12
I (P+G.K)	0.96 ± 0.06	10.46 ± 1.48	0.80 ± 0.17
J (B+M)	0.97 ± 0.06	9.96 ± 0.85	0.60 ± 0.20
P – Values	<0.0001	<0.0001	0.0001
F – Values	27.55	8.491	7.187

Study was done in triplicate, ANOVA, followed by Tukey's multiple comparison tests. NC = Negative control, PC= Positive control, PROP and P = Propranolol, MOT and M = Motherwort, BUG and B= Bugleweed, G.K = Garcinia kola

Table 4a. Summary of Group Comparison of Tukey Multiple Comparison Test; Mean ± SD Thyroid hormones for the controls and test groups at 30 Days of treatment

Groups	T3 (ng/ml)	T4 (ug/dl)	TSH (ulu/ml)
Group A vs Group B	***	***	ns
Group A vs Group C	ns	*	ns
Group A vs Group D	ns	***	ns
Group A vs Group E	*	***	ns
Group A vs Group F	*	***	ns
Group A vs Group G	ns	***	*
Group A vs Group H	ns	***	ns
Group A vs Group 1	ns	*	ns
Group A vs Group J	ns	ns	ns
Group B vs Group C	***	ns	*
Group B vs Group D	***	ns	**
Group B vs Group E	***	ns	ns
Group B vs Group F	***	ns	***
Group B vs Group G	***	ns	***
Group B vs Group H	***	ns	*
Group B vs Group 1	***	*	ns
Group B vs Group J	***	*	ns
Group C vs Group D	ns	ns	ns
Group C vs Group E	ns	ns	ns
Group C vs Group F	ns	ns	ns
Group C vs Group G	ns	ns	ns
Group C vs Group FI	ns	ns	ns

Key: A =Negative Control, B = Positive Control, C= Propranolol, D= Motherwort, E= Bugleweed, F= Garcinia kola, G= Combination of Propranolol and Bugleweed, H= Combination of Propranolol and Motherwort, I= Combination of Propranolol and Garcinia kola, J= Combination of Bugleweed and Motherwort.

thyroid disease such as hyperthyroidism, [21]. There were significant increases (*p<.01*) in the levels of T3 and T4 and decreases in TSH levels for days 14, 30 and 60 of the experiment after rats were exposed to cyanide. Significant decreases (*<.05*) in the thyroid functions of the rats treated with the herbal supplements for 14, 30 and 60 days was observed, as shown in Tables 1, 3 and 5 respectively. These reductions may be due to inhibition of the infiltration by the herbal supplements.

The increases in the T_3 and T_4 levels is an indication that the synthetic function of the thyroid might have been impaired since the evaluation of TSH level is a good index for assessing the metabolic ability of the thyroid. Most of the plasma T_4 and T_3 are protein bound to an α- globulin, thyroxine – binding globulin and to a lesser extent to transthyretin previously called pre –albumin, the free unbound fractions were in physiologically active form, which regulates the TSH secretion from the anterior pituitary [22]. The lower levels in the thyroid function tests (T_3, T_4) by the supplements (Bugleweed), shown in the Post Hoc test (Tables 2, 4 and 6), may be due to inhibition of the binding of antibodies. Prior to secretion of thyroid

Table 4b. Summary of group comparison of tukey multiple comparison test; mean ± sd thyroid hormones for the controls and test groups at 30 days of treatment

Groups	T3 (ng/ml)	T4 (ug/dl)	TSH (ulu/ml)
Group C vs Group 1	ns	ns	ns
Group C vs Group J	ns	ns	ns
Group D vs Group E	ns	ns	ns
G'cup D vs Group F	ns	ns	ns
Group D vs Group G	ns	ns	ns
Group D vs Group H	ns	ns	ns
Group D vs Group 1	ns	ns	ns
Group D vs Group J	ns	ns	ns
Group E vs Group F	ns	ns	ns
Group E vs Group G	ns	ns	ns
Group E vs Group H	ns	ns	ns
Group E vs Group 1	ns	ns	ns
Group E vs Group J	ns	ns	ns
Group F vs Group G	ns	ns	ns
Group F vs Group H	ns	ns	ns
Group F vs Group 1	ns	ns	ns
Group F vs Group J	ns	ns	*
Group G vs Group H	ns	ns	ns
Group'G vs Group i	ns	ns	*
Group G vs Group J	ns	ns	**
Group H vs Group 1	ns	ns	ns
Group H vs Group J	ns	ns	ns
Group I vs Group J	ns	ns	ns

Key: A =Negative Control, B = Positive Control, C= Propranolol, D= Motherwort, E= Bugleweed, F= Garcinia kola, G= Combination of Propranolol and Bugleweed, H= Combination of Propranolol and Motherwort, I= Combination of Propranolol and Garcinia kola, J= Combination of Bugleweed and Motherwort.

Table 5. Mean ± SD thyroid hormones of cyanide induced hyperthyroid rats after 60 days of treatment with herbal supplements

Groups	T3 (ng/ml)	T4 (µg/dl)	TSH (µiu/ml)
A (NC)	0.80 ± 0.20	7.67 ± 2.31	1.10 ± 0.27
B (PC)	2.80 ± 0.26	16.23 ± 2.48	0.10 ± 0.01
C (PROP)	0.87 ± 0.29	11.33 ± 1.66	0.53 ± 0.15
D (MOT)	0.70 ± 0.10	9.16 ± 3.50	1.00 ± 0.20
E (BUG)	1.03 ± 0.21	11.93 ± 0.92	0.80 ± 0.17
F (G.K)	0.93 ± 0.32	10.33 ± 1.80	1.66 ± 0.58
G (P+B)	0.70 ± 0.01	10.90 ± 0.72	1.33 ± 0.58
H (P+M)	0.60 ± 0.01	11.60 ± 2.33	1.00 ± 0.01
I (P+G.K)	0.76 ± 0.15	9.13 ± 3.00	0.50 ± 0.17
J (B+M)	0.90 ± 0.10	9.40 ± 1.63	0.66 ± 0.25
P – Values	< 0.0001	0.011	0.0002
F – Value	32.23	3.388	6.59

Study was done in triplicate, ANOVA, followed by Tukey's multiple comparison tests. NC = Negative control, PC= Positive control, PROP and P = Propranolol, MOT and M = Motherwort, BUG and B = Bugleweed, G.K = Garcinia kola

hormones, iodide is actively taken up by the thyroid gland under the control of TSH via the sodium symporter [23,24]. Uptake of these were blocked by thiocyanate and perchorate. The concentration of iodide was more in plasma and is rapidly converted to iodine within the thyroid gland which was catalyzed by thyroid peroxidase. The iodination of tyrosine residues in glycoprotein, thyroglobulin, will take place which will form mono – iodotyrosine and di – iodotyrosine that was mediated by the enzymes thyroid peroxidase which is inhibited by carbimazole and propylthiouracil. Iodotyrosines are coupled to form T_4 and T_3 which were stored in the lumen of thyroid follicular cells [25]. Normally much more T_4 than T_3 are synthesized, but, if there are adequate supply of iodide the ratio of T_3 to T_4 in the gland increases. The thyroid hormones still incorporated in thyroglobulin are stored in the colloid of the thyroid follicle [26]. Endocytosis and phagocytosis are involved in the uptake of

Table 6a. Summary of Group Comparison of Tukey Multiple Comparison Test; Mean thyroid hormones for the controls and test groups at 60 Days

Groups	T3 (ng/ml)	T4 (ug/dl)	TSH (ulu/ml)
Group A vs Group B	***	*	*
Group A vs Group C	ns	ns	ns
Group A vs Group D	ns	ns	ns
Group A vs Group E	ns	ns	ns
Group A vs Group F	ns	ns	ns
Group A vs Group G	ns	ns	ns
Group A vs Group H	ns	ns	ns
Group A vs Group 1	ns	ns	ns
Group A vs Column J	ns	ns	ns
Group B vs Group C	***	ns	ns
Group B vs Group D	***	*	*
Group B vs Group E	***	ns	ns
Group B vs Group F	***	ns	***
Group B vs Group G	***	ns	**
Group B vs Group H		ns	*
Group B vs Group 1	*	*	ns
Group B vs Column J	***	*	ns
Group C vs Group D	ns	ns	ns

Key: A =Negative Control, B = Positive Control, C= Propranolol, D= Motherwort, E= Bugleweed, F= Garcinia kola, G= Combination of Propranolol and Bugleweed, H= Combination of Propranolol and Motherwort, I= Combination of Propranolol and Garcinia kola, J= Combination of Bugleweed and Motherwort.

Table 6b. Summary of group comparison of tukey multiple comparison test; mean thyroid hormones for the controls and test groups at 60 days

Groups	T3 (ng/ml)	T4 (ug/dl)	TSH (ulu/ml)
Group C vs Group E	ns	ns	ns
Group C vs Group F	ns	ns	**
Group C vs Group G	ns	ns	ns
Group C vs Group F	ns	ns	ns
Group C vs Group 1	ns	ns	ns
Group C vs Column J	ns	ns	ns
Group D vs Group E	ns	ns	ns
Group D vs Group F	ns	ns	ns
Group D vs Group G	ns	ns	ns
Group D vs Group H	ns	ns	ns
Group D vs Group 1	ns	ns	ns
Group D vs Column J	ns	ns	ns
Group E vs Group F	ns	ns	ns
Group E vs Group G	ns	ns	ns
Group E vs Group H	ns	ns	ns
Group E vs Group 1	ns	ns	ns
Group E vs Column J	ns	ns	ns
Group F vs Group G	ns	ns	ns
Group F vs 6Group H	ns	ns	ns
Group F vs Group 1	ns	ns	**
Group F vs Column J	ns	ns	*
Group G vs Group H	ns	ns	ns
Group G vs Group 1	ns	ns	ns
Group G vs Column J	ns	ns	ns
Group H vs Group 1	ns	ns	ns
Group H vs Column J	ns	ns	ns
Group 1 vs Column J	ns	ns	ns

Key: A =Negative Control, B = Positive Control, C= Propranolol, D= Motherwort, E= Bugleweed, F= Garcinia kola, G= Combination of Propranolol and Bugleweed, H= Combination of Propranolol and Motherwort, I= Combination of Propranolol and Garcinia kola, J= Combination of Bugleweed and Motherwort.

thyroglobulin by follicular cells. Proteolytic enzymes release T4 and T3 into the bloodstream; TSH stimulates this process, while iodide inhibits it. Thyroid hormones bind to plasma proteins right away. Monoiodotyrosine and di-iodotyrosine are both released at the same time, hence the iodine is reused. Specific enzymes regulate each process, and a congenital defect in any of these enzymes can result in hyperthyroidism. The findings of this study indicate that the administration of supplements in cyanide induced rats exhibits a significant protection on the thyroid gland.

4. CONCLUSION

Cyanide exposure in rats caused hyperthyroidism, but administration of some herbal supplements ameliorated the effect of cyanide. This indicates that these supplements could be useful for the treatment of cyanide poisoning and therefore requires further studies, especially on the molecular mechanisms of their effects.

NOTE

The study highlights the efficacy of "name" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

ETHICAL APPROVAL

Experimental Animal Care and Ethics Committees, Ministry of Agriculture. Rivers State with permit number MA/VET/570/01.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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