Original Research Article

Assessment of the effects of some Herbal Supplements on some Inflammatory and Hepatic markers of Cyanide – Induced Hyperthyroid Female Albino Rats

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ABSTRACT

Aim: To assess the effects of some herbal supplements on some inflammatory and hepatic markers of cyanide – induced hyperthyroid female Albino Rats.

Study design: Experimental study

Place and Duration of Study: Department of Animal and Environmental Sciences, 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 15^{th}, 31^{st}, and 61^{th} 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 inflammatory and hepatic markers. Laboratory estimations of C reactive protein and tissue necrosis factor alpha were analyzed using the ELISA technique, while liver enzymes were analyzed using spectrophotometric method. 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 the levels of C- reactive protein ($p<.01$), Tumor neurosis factor –alpha ($p<.01$) were significantly lower in the treated rats compared to the positive control group. The activities of the liver enzymes, AST ($p<.01$), ALT ($p<.01$) and ALP ($p<.01$) were significantly reduced indicating a decrease in the impairment associated with the chemical alteration of the follicular cells, inflammation and non- toxicity of the herbal supplements and extract at therapeutic doses.

Conclusion: The herbal supplements and extract have the ability to reduce the inflammatory effect of hyperthyroidism, therefore, further studies are recommended.

Keywords: Herbal supplements, inflammatory, hepatic markers, cyanide, hyperthyroid, female Albino Rats.

1. INTRODUCTION

There is mounting evidence that environmental exposures, particularly chemical exposures, should be regarded potential thyroid disease risk factors. Thyroid disruptors such as pesticides, herbicides, and fungicides should be regarded potential risk factors for thyroid illness [1,2]. However, there is yet no proven cure for hyperthyroidism, the treatment modalities that are available for the disease can alleviate the symptoms such as heart problems, brittle bones, eye problems, and red swollen skin.

Cyanide is a poisonous, fast-acting chemical with a long history. In 1786, hydrogen cyanide was separated from Prussian blue dye, and in 1800, cyanide was recovered from almonds for the first time. It can be found in the form of a gas, hydrogen cyanide, or a salt, potassium cyanide. Structure fires, industrial exposures, medicinal exposures such as sodium nitroprusside, and certain foods are all potential sources of cyanide poisoning. In domestic countries, the most common cause of cyanide poisoning is domestic fires. Toxic levels of cyanide may be present in patients who receive prolonged infusions of sodium nitroprusside [3]. Intravenous and inhalation cyanide exposure results in a faster start of signs and symptoms than oral cyanide exposure. Because the first two routes provide rapid diffusion into the bloodstream, this is the case. Goiter, pancreatic diabetes, and a variety of neurological problems have all been linked to long-term exposure to cyanide and/or its major metabolite, thiocyanate. However, there is relatively little information in the literature about these drugs' hepatotoxic and nephrotoxic effects.

Without trustworthy data, the World Health Organization believes that almost 80% of the world's population is reliant on traditional medicine. This is especially true when a developing country strives to provide universal health coverage to its citizens. Traditional medicine is also reported to have a higher level of acceptance among people in poor nations, partly due to the inaccessibility of orthodox pharmaceuticals, but the main contributing aspect is that it blends easily into the socio-cultural life of the people whose culture it is deeply based [4]. The use of plant-based materials including herbal or natural health care products with supposed health benefits are increasing in developed countries [5]. This brings some risks of toxicity and other effects on human health, despite the safe image of herbal remedies. There are claims that herbal supplements are better therapies for hyperthyroidism or complications that arise as a result of the disease, mainly due to the complex etiology of the disease [6]. Currently, the drugs used for the treatment of this disease have been reported to have adverse side effects [1], 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.

A large number of herbs are known to possess anti-thyroid activity. Many different phytoconstituents are known to be present in herbs and these phytoconstituents have different mechanism of action. Various herbal plants are available in the market for the management of hyperthyroidism. These includes Bugleweed (*Lycopus virginicus*), Lemon (*Mellisa officinal*), Motherwort (*Leonurus cardiac*), Gromwell (*Lithospermum ruderale*), Rosemaay (*Rosmarinus officinalis*), Sage (*Salvia officinalis*) and *Garcinia kola* (Bitter cola). For this study, three medicinal supplements were considered. 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 a forest tree native to Sub-Saharan Africa that is widely cultivated. It's been dubbed a "wonder plant" since practically every portion of it has been discovered to have medicinal properties. The seed is utilised in traditional hospitality, cultural, and social ceremonies as a masticatory. 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.

Moreover, there is a growing interest in the use of herbal supplementation for the treatment and management of human diseases including hyperthyroidism, because the herbal supplements are credited with medicinal efficacies [7,8]. However, there is very scanty scientific and evidence-based evaluation of the anti- hyperthyroidism effects of the herbal supplement such as Bugleweed, Motherwort and *Garcinia Kola* used in Nigeria. Therefore, this study assessed the effects of some herbal supplements on some inflammatory and hepatic markers of cyanide – induced hyperthyroid 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. [9].

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 Paget and Barnes 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 Guidelines [10]. 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 [10] 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 [10] 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 [10] 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 [10] Guidelines, this dosage was 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 was dissolved in 2 x 0.5/0.009 = 111.1 ml of diluent.

2.5 Induction of Hyperthyroidism and Treatment with Herbs

From a previously conducted pilot toxicity study, 2.4 mg/kg of potassium hexacyanoferrate III salt was used to induce hyperthyroidism in rats, Adeniyi et al. [11]. After induction of hyperthyroidism, rats were treated with the herbal supplements (Bugleweed and Motherwort), *Garcinia kola* and Propranolol, 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 fours (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 C – Reactive Protein (CRP)

Method: ELISA using Rat specific (CRP) ELISA kit [12]

2.8.2 Estimation of Tumor Necrosis Factor – Alpha

Method: ELISA using Rat specific (TNF- α) ELISA kit [12]

2.8.3 Estimation of Aspartate Transaminases

Method: Spectrophotometric method

2.8.4 Estimation of Alanine Transaminase

Method: Spectrophotometric method

2.8.5 Estimation of Alkaline Phosphatase

Method: Spectrophotometric method

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

Table 1: Mean ± SD Inflammatory Markers of Cyanide – Induced Hyperthyroid Rats According to Groups after 14 Days of Treatment with Drug, Herbal Supplements and Extract.

Groups	CRP (mg/l)	TNF- α (pg/ml)
A (NC)	5.67± 1.15	13.57±2.48
B (PC)	13.00±1.73	20.43±0.11
C (PROP)	9.33±0.56	17.73±1.67
D (MOT)	9.33±0.23	15.13±0.29
E (BUG)	6.33±0.57	12.07±0.06
F (G.K)	5.06±0.12	10.53±0.06
G (P+B)	6.00±0.01	11.40±0.52
H (P+M)	3.67±1.12	12.00±0.01
I (P+G.K)	5.06±0.11	11.66±0.11
J (B+M)	3.83±0.29	11.60±0.01
p – Values	<0.0001	<0.0001
F – Values	*41.79*	*33.72*

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, CRP = C – Reactive Protein, TNF – α = Tumour Necrosis Factor-alpha

Table 2a: Summary of Group Comparison of Tukey Multiple Comparison Test. Mean -±SD for Inflammatory Markers of the controls and test groups at 14 Days

Groups	CRP (mg/L)	TNF- α (pg/ml)
Group A vs Group B	***	***
Group A vs Group C	***	***
Group A vs Group D	***	ns
Group A vs Group F	Ns	*
Group B vs Group C	***	ns
Group B vs Group D	**	***
Group B vs Group E	***	***
Group B vs Group F	***	***
Group B vs Group G	***	***
Group B vs Group F	***	***
Group B vs Group I	***	***
Group B vs Group J	***	***
Group C vs Group E	***	***
Group C vs Group F	***	***
Group C vs Group G	***	***

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 for Inflammatory Markers of the controls and test groups at 14 Days

Groups	CRP (mg/L)	TNF- α (pg/ml)
Group C vs Group H	***	***
Group C vs Group I	***	***
Group C vs Group J	***	***
Group D vs Group E	***	***
Group D vs Group F	***	***
Group D vs Group G	***	***
Group D vs Group H	***	***
Group D vs Group I	***	***
Group D vs Group J	***	***
Group E vs Group H	*	ns
Group E vs Group J	*	ns
Group G vs Group H	*	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: Mean ± SD Inflammatory Markers of Cyanide – Induced Hyperthyroid Rats after 30 Days Treatment with Drug, Herbal Supplements and Extract.

Groups	CRP (mg/l)	TNF –α (pg/ml)
A (NC)	1.00 ± 0.01	11.90 ± 1.73
B (PC)	11.27 ± 1.10	20.40 ± 0.44
C (PROP)	1.07 ± 0.12	11.40 ± 0.44
D (MOT)	1.07 ± 0.11	12.70 ± 2.46
E (BUG)	0.96 ± 0.06	11.20 ± 1.04
F 9G.K)	1.40 ± 0.17	12.23 ± 2.04
G (P+B)	1.00 ± 0.01	14.53 ± 4.47
H (P+M)	1.03 ± 0.06	9.60 ± 0.79
I (P+G.K)	0.86 ± 0.11	7.33 ± 4.04
J (B+M)	1.47 ± 0.25	9.16 ± 1.04
P – Values	<0.0001	0.0001
F – Values	23.01	7.201

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 4: Summary of Group Comparison of Tukey Multiple Comparison Test. Mean ± SD for Inflammatory Markers of the controls and test groups at 30 Days

Groups	CRP (mg/L)	TNF-a (pg/ml)
Group A vs Group B	***	**
Group B vs Group C	***	**
Group B vs Group D	***	*
Group B vs Group E	***	**

Group B vs Group F	***	**
Group B vs Group G	***	ns
Group B vs Group F	***	***
Group B vs Group I	***	***
Group B vs Group J	***	***
Group G vs Group I	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 Inflammatory Markers of Cyanide - Induced Hyperthyroid Rats after 60 Days of Treatment with Drug, Herbal Supplements and Extract.

Groups	CRP (mg/l)	TNF – α (pg/ml)
A (NC)	0.73 ± 0.06	10.36 ± 0.66
B (PC)	10.60 ± 0.46	20.53 ± 0.31
C (PROP)	0.67 ± 0.12	10.33 ± 0.68
D (MOT)	0.70 ± 0.17	12.23 ± 2.76
E (BUG)	0.76 ± 0.06	9.46 ± 11.70
F (G.K)	1.06 ± 0.23	11.70 ± 1.95
G (P+B)	0.70 ± 0.01	13.90 ± 4.51
H (P+M)	0.70 ± 0.17	7.43 ± 3.63
I (P+G.K)	0.60 ± 0.01	6.40 ± 5.62
J (B+M)	1.36 ± 032	10.16 ± 015
P – Values	< 0.0001	0.0011
F – Values	64.72	5.192

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 6: Summary of Group Comparison of Tukey Multiple Comparison Test. Mean ± SD for Inflammatory Markers of the controls and test groups at 60 Days

Groups	CRP (mg/L)	TNF-a (pg/mL)
Group A vs Group B	***	*
Group A vs Group J	*	ns
Group B vs Group C	***	*
Group B vs Group D	***	ns
Group B vs Group E	***	**
Group B vs Group F	***	*
Group B vs Group G	***	ns
Group B vs Group F	***	***
Group B vs Group I	***	***
Group B vs Group J	***	*
Group C vs Group J	*	ns
Group D vs Group J	*	ns
Group G vs Column J	*	ns
Group H vs Column J	*	ns

Group 1 vs Column J	**	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 7: Mean ± SD Levels of Liver Variables of Cyanide – Induced Hyperthyroid Rats According to Groups after 14 Days of Treatment.

Groups	AST (IU/L)	ALT (IU/L)	ALK. PHOS (IU/L)
A (NC)	11.67 ± 0.58	9.67 ± 0.58	25.33 ± 2.31
B (PC)	25.67 ± 2.31	27.00 ± 0.01	47.33 ± 2.89
C (PROP)	17.33 ± 2.31	22.33 ± 0.58	46.33 ± 2.88
D (MOT)	33.00 ± 5.19	35.67 ± 9.23	42.67 ± 2.89
E (BUG)	28.00 ± 3.46	24.33 ± 2.88	41.66 ± 2.89
F (G.K)	23.67 ± 0.58	24.00 ± 1.73	41.66 ± 1.15
G (P+B)	66.00 ± 6.12	32.00 ± 17.32	44.66 ± 2.31
H (P+M)	92.23 ± 2.88	34.33 ± 6.35	49.66 ± 8.08
I (P+G.K)	124.00 ± 24.24	50.00 ± 8.66	42.00 ± 0.01
J (B+M)	157.00 ± 36.37	72.33 ± 21.93	66.00 ± 5.19
P – Values	< 0.0001	< 0.0001	0.0724
F – Values	11.3	9.041	2.143

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, CRP = C – Reactive Protein, TNF – α = Tumour Necrosis Factor-Alpha. QC Values for AST = 35 IU/L, ALT = 20 IU/L, ALK. PHOS = 50 IU/L.

Table 8: Summary of Group Comparison of Tukey Multiple Comparison Test. Mean ± SD liver variables for the controls and test groups at Day 14.

Groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Group A vs Group F	*	ns	ns
Group A vs Group I	**	**	ns
Group A vs Group J	***	***	ns
Group B vs Group I	**	ns	ns
Group B vs Group J	***	***	ns
Group C vs Group I	**	ns	ns
Group C vs Group J	***	***	ns
Group D vs Group I	*	ns	ns
Group D vs Group J	***	**	ns
Group E vs Group I	**	ns	ns
Group E vs Group J	***	***	ns
Group F vs Group I	**	ns	ns
Group F vs Group J	***	***	ns
Group G vs Group J	*	**	ns
Group F vs Group J	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 9: Mean ± SD Liver Variables of Cyanide - Induced Hyperthyroid Rats According to Groups after 30 Days of Treatment with Drug, Herbal Supplements and Extract.

Groups	AST (IU/L)	ALT (IU/L)	ALK. PHOSP (IU/L)
A (NC)	10.00 ±0.01	9.33 ± 0.58	23.33 ± 2.08
B (PC)	20.67 ± 4.04	23.00 ± 0.01	43.00 ± 2.65
C (PROP)	15.00 ± 4.58	12.67 ± 2.51	23.33 ± 5.77
D (MOT)	10.33 ± 10.96	17.00 ± 12.12	29.00 ± 5.77
E (BUG)	14.00 ± 4.58	9.33 ± 6.35	16.00 ± 1.73
F (G.K)	12.00 ± 6.92	4.33 ± 3.21	23.00 ± 1.73
G (P+B)	15.00 ± 4.58	12.66 ± 2.52	20.00 ± 0.01
H (P+M)	5.00 ± 1.73	3.33 ± 1.52	13.33 ± 2.31
I (P+G.K)	10.00 ± 3.00	2.67 ± 0.57	15.33 ± 4.16
J (B+M)	13.00 ± 3.00	15.00 ± 3.46	31.66 ± 5.13
P – Values	0.1063	0.0006	<0.0001
F – Values	1.928	5.617	13.18

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

Table 10: Summary of Group Comparison of Tukey Multiple Comparison Test. Mean liver and renal variables for the controls and test groups treated for 30 Days

Groups	AST (IU/L)	ALT (IU/L)	ALP PHOS (IU/L)
Group A vs Group B	ns	ns	***
Group B vs Group C	ns	ns	***
Group B vs Group D	ns	ns	*
Group B vs Group E	ns	ns	***
Group B vs Group F	ns	*	***
Group B vs Group G	ns	ns	***
Group B vs Group H	*	**	***
Group B vs Group I	ns	*	***
Group D vs Group I	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 11: Mean ± SD Liver Variables of Cyanide Induced Hyperthyroid Rats after 60 Days of Treatment with Drug, Herbal Supplements and Extract.

Groups	AST (IU/L)	ALT (IU/L)	ALK.PHOS (IU/L)
A (NC)	8.00 ± 1.73	5.00 ± 0.10	21.00 ± 3.46
B (SPC)S	16.66 ± 2.52	15.00 ± 3.47	39.00 ± 1.00
C (PROP)	4.00 ± 0.02	5.33 ± 4.93	18.66 ± 2.31
D (MOT)	5.00 ± 1.73	3.00 ± 1.73	14.67 ± 4.62
E (BUG)	5.00 ± 1.73	2.33±5.70	15.33 ± 2.88
F (G.K)	8.00 ± 1.73	3.33 ± 1.52	14.00 ± 2.00
G (P+B)	4.00 ± 0.01	3.00 ± 1.73	15.00 ± 3.46
H (P+M)	8.00 ± 1.73	5.00 ± 0.01	21.00 ± 3.46
I (P+G.K)	6.00 ± 1.73	4.00 ± 1.73	19.66 ± 2.08

J (B+M)	6.00 ± 3.46	2.66 ± 0.58	31.66 ± 5.13
P – Values	< 0.0001	< 0.0001	< 0.0001
F – Values	11.41	8.57	18.82

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 12: Summary of Group Comparison of Tukey Multiple Comparison Test. Mean liver variables for the controls and test groups treated at Day 60

Groups	AST (IU/L)	ALT (IU/L)	ALP PHOS (IU/L)
Group A vs Group B	***		
Group A vs Column J	Ns	ns	*
Group B vs Group C	***	***	***
Group B vs Group D	***	***	***
Group B vs Group E	***	***	***
Group B vs Group F	***	***	***
Group B vs Group G	***	***	***
Group B vs Group F	***	***	***
Group B vs Group I	***	***	***
Group B vs Column J	***	***	ns
Group C vs Column J	Ns	ns	**
Group D vs Column J	Ns	ns	***
Group E vs Column J	Ns	ns	***
Group F vs Column J	Ns	ns	***
Group G vs Column J	ns	ns	***
Group H vs Column J	ns	ns	*
Group 1 vs Column J	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.

The parameters used to assess the inflammation in this study were CRP and Tumor Necrosis TNF-α. TNF- α is a cytokine produced by activated macrophages and monocytes which plays a number of important roles in the mechanism of defense while CRP is widely known as a sensitive marker of low-grade inflammation. This study demonstrated that the administration of cyanide caused inflammation at the site of the thyroid thereby causing hyperthyroidism. The data from this study showed that the levels of the inflammatory markers were significantly higher in the hyperthyroid control group compared to the treated groups for the three periods of treatments (Tables 1, 3 and 5) and this agrees with the work of Tzoulaki et al. [13], who reported that acute phase reactants are usually produced during inflammations like hyperthyroidism. However, a strong relation between thyroid hormone and haemodynamic of the heart has been established and has been found to be associated with hyperthyroidism. Moreover, high sensitive CRP has been found to be associated with atherosclerosis and various diseases of the heart vessels [13]. The levels of the inflammatory markers were significantly reduced in the groups treated with the herbal supplements, compared to the hyperthyroid group for the three periods of treatments (Tables 2(a & b),4 and 6). This is likely owing to the inhibitory effects of the phytonutrient saponin in

herbal supplements on the start of inflammation. Saponin showed strong anti-inflammatory action, which could be mediated by inhibiting the release and synthesis of the molecules implicated in inflammation. The biological activities of saponins from medicinal plants have been linked to their amphiphilic nature, which aids in exhibiting these activities via their ability to intercalate into the plasma membrane, resulting in changes in membrane fluidity, which then affect membrane function, causing cellular responses.

The parameters used to assess the liver damage/ injury were aspartate transaminases, Alaine transaminases and alkaline phosphatase. The liver is a vital organ of immense importance. It is involved in the maintenance of metabolic functions and detoxification of endogenous and exogenous matters like exposure to toxins [14]. The study demonstrated that cyanide causes detrimental changes in the liver by inducing toxicity upon administration of 2.4mg/kg of it to rats.

Liver dysfunction in hyperthyroidism can be due to a number of factors, including the disease itself, other autoimmune disease or infection and anti- hyperthyroid drugs such propranolol [14]. This study also evaluated the effect of the herbal supplementation on the activities of the liver enzymes. In this study the pattern of results was observed that the hyperthyroid group had significantly higher activities of AST, ALT and ALP than the treated group in the three periods of treatments (Tables 7, 9 and 11), indicating a damage to the liver cells. The increased levels of serum enzymes indicate cellular leakage and a loss of functional integrity of the liver's cell membrane. This is because the transaminases (AST and ALT) are found in the periportal hepatic cells, whereas the alkaline phosphatase is found in the cells lining the liver's biliary duct. These enzymes are released in hepatic damages due to the loss of hepatocyte structural integrity and leakage hence known as biomarkers of hepatic damage [16]. The inflammation in the liver leads to an increase in the activities of the liver enzymes (Tables 7, 9 and 11). The levels are seen as indicator of hepatic dysfunction due to cyanide-induced hyperthyroidism [17]. The assay of these liver enzymes has been seen as a simple method of evaluating the anti-hyperthyroid activity of any target drugs. There was as significant difference ($p<.05$) in the enzyme levels when all the levels in the different groups was compared with the control groups. The levels of the enzymes were significantly reduced in the rats that were treated with the herbal supplements (Day 60) (Table 11 and 12). Thus, the herbal supplementation used in this study were able to reverse the liver impairments that are associated with cyanide-induced hyperthyroidism [18]. The reduction in the activities of these enzymes also indicated that therapeutic dose and these herbal supplements are not toxic to the liver and therefore do not pose any threat to the integrity of the liver. Similar findings have been reported by other researchers using other herbal supplements [19,14,20].

The lower levels in the serum enzymes by the herbal supplements may be due to the prevention of the leakage of the intracellular enzymes since *garcinia Kola* is known to be a membrane stabilizer as stated by Iwu et al. [21]. This finding also agrees with the study of Scappaticcio et al. [20] which stated that serum levels of hepatic enzymes return to normal with the healing of hepatic parenchyma and regeneration of hepatocytes. Saro & Tse [18] stated that the efficacy of any hepato- protective drug can be based on either the capacity to reduce the harmful effect or the ability to restore the cells to normal hepatic physiology after an attack by a toxin.

4. CONCLUSION

The herbal supplements and extract have the ability to reduce the inflammatory and hepatotoxic effects of hyperthyroidism. Further studies are required to investigate the mechanism by which these herbal supplements reduce the chemical induced hyperthyroidism.

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.

AUTHORS' CONTRIBUTIONS

Authors NEO, TDG and EI designed the study and supervised the work, while author OBNC wrote the protocol, and wrote the first draft of the manuscript, managed the analyses of the study and managed the literature searches. All authors read and approved the final manuscript.

COMPETING INTERESTS DISCLAIMER:

AUTHORS HAVE DECLARED THAT NO COMPETING INTERESTS EXIST. THE PRODUCTS USED FOR THIS RESEARCH ARE COMMONLY AND PREDOMINANTLY USE PRODUCTS IN OUR AREA OF RESEARCH AND COUNTRY. THERE IS ABSOLUTELY NO CONFLICT OF INTEREST BETWEEN THE AUTHORS AND PRODUCERS OF THE PRODUCTS BECAUSE WE DO NOT INTEND TO USE THESE PRODUCTS AS AN AVENUE FOR ANY LITIGATION BUT FOR THE ADVANCEMENT OF KNOWLEDGE. ALSO, THE RESEARCH WAS NOT FUNDED BY THE PRODUCING COMPANY RATHER IT WAS FUNDED BY PERSONAL EFFORTS OF THE AUTHORS.

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