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Pineapple juice supplementation activates thyroid gland and attenuates hyperlipidemia in rats

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Abstract

This study aimed to assess the antioxidant activity of the pineapple crude juice extracted from the whole fruit, and its effect in stimulating thyroid hormones synthesis in hypothyroid rats, as well as anticipating the concomitant hyperlipidemia. Male Swiss albino rats were divided into: normal (euthyroid) controls; normal rats orally administrated with pineapple juice (0.75 ml/100g body weight) for 8 consecutive weeks; hypothyroid rats administrated methimazole (0.02% w/v) in drinking water for 15 days; and treated hypothyroid rats orally administrated with methimazole then pineapple juice for 8 weeks. *In vitro* antioxidant studies demonstrated that pineapple juice has high scavenging activity for nitric oxide and α,α_2-diphenyl-β-picrylhydrazyl radicals, a high ferric reducing antioxidant power and an inhibitory effect on lipid peroxidation. Supplementation of euthyroid rats with pineapple juice for 8 weeks reduced the body weight and elevated serum T_3 and T_4 levels, compared to normal controls. While, supplementing hypothyroid rats with the pineapple juice normalized serum levels of T_3, free cholesterol and its percentage of esterification, LDL-C, HDL-C, atherogenic index and LCAT activity, as well as serum, heart and hepatic MDA concentration and GPX activity. The histological examination of thyroid tissue sections of hypothyroid supplemented rats revealed an improvement in the cellular architecture of the thyroid gland epithelium lining the follicles and partial filling of the follicular lumen with colloid. Pineapple juice attenuates the excessive methimazole induced oxidative stress and consequent hyperlipidemia, also, activates the thyroid gland functions, suggesting its benefit as therapeutic supplement or as an adjunct in hypothyroidism therapy.

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Introduction

Pineapple (*Ananas comosus)* is the most economically important plant in the family *Bromeliaceae*. It belongs to the subfamily *Bromelioideae,* order *Bromeliales,* genus *Ananas* and species *Comosus* (Geo and Freddy, 2003). Pineapple fruit has a high nutritional value, 100g of a fresh fruit contain: vitamins A (58IU),C (47.8mg), niacin (0.5mg), E (0.02mg), riboflavin (0.018mg), folates (18 µg) and K (0.07µg),as well as minerals such as calcium (24mg), magnesium (12mg), phosphorus (8mg), manganese(0.927mg), iron (0.2mg), copper(0.1mg), zinc(0.1mg), and selenium(0.1ug). The edible part of the pineapple fruit (pulp) is made up of 85% water, 14%sugarof which two-thirds are sucrose and the rest are glucose and fructose, 0.6% fiber, 0.5% protein, 0.4% ash and 0.1% fat(Siong, 1982).The medicinal value of pineapple is attributed mainly to bromelain, a 95% mixture of proteases. The highest concentration of bromelain is found in the ripe pineapple fruit pulp and stem (Chobotova *et al.*, 2010). Bromelain consists mainly of sulfhydryl-containing proteases with a small amount of other enzymes, such as peroxidase, acid phosphatase, amylase, glucosidases and cellulases. Although these enzymes differ in their substrate specificity, molecular mass, isoelectric point and pH optimum, bromelain is internationally classified as a single entity enzyme (Maurer, 2001). It also contains escharase (a non proteolytic component), several protease inhibitors, glycoproteins, carbohydrates and organically bound calcium (Bhattacharyya, 2008). A wide range of therapeutic benefits has been reported for bromelain, such as improving cardiovascular and circulatory diseases (Nieper, 1978), acceleration of wound healing(Kelly, 1996)‚reversible inhibition of platelet aggregation, anti-edema (MacKay *et al.*, 2003), antithrombotic and fibrinolytic activity (Errasti *et al.*, 2016). As an adjunct in cancer therapy, bromelain acts as an immunomodulator by raising the impaired immunocytotoxicity of monocytes against tumor cells and by inducing the cytokines production (Bhattacharyya, 2008). Hypothyroidism is an endocrine disorder that may occur as a result of primary gland failure or insufficient thyroid gland stimulation by the hypothalamus or the pituitary gland. Clinical examination findings associated with hypothyroidism include goiter, brittle hair, dry skin, peripheral oedema and bradycardia, while laboratory findings include hyponatraemia, hypercapnia, hypoxia, normocytic anaemia, elevated creatine kinase, hyperprolactinaemia and hyperlipidemia (Gaitonde *et al.*, 2012). In hypothyroid patients, alleviation of the symptoms could be accomplished by oral administration of synthetic levothyroxine, as a lifelong therapy. However, the patients usually suffer over-replacement, which may result in an increased risk of cardiovascular disease, osteoporosis and subclinical liver damage.

The present work was undertaken to assess the therapeutic benefit of the use of the crude pineapple juice from the whole fruit, as a food supplement, in activating the thyroid gland and anticipating the complications associated with hypothyroidism in a rat model. Histological examination of the thyroid gland was concurrently done to emphasize the biochemical findings. In addition, *in vitro* antioxidant studies of the pineapple juice were carried out.

Materials and methods

Preparation and administration of the pineapple juice

A ripe pineapple fruit was peeled and the whole fruit (pulp and stem) was cut into large pieces then squeezed using an electric squeezer. The fresh crude juice was filtered, aliquoted and stored at -20^{o}C until use. Undiluted pineapple juice was daily administered to rats by intragastric tubing for 8 consecutive weeks at a dose level of 0.75 ml/100 g b.w (equivalent to 525 ml/70 kg b.w. in human).

In vitro antioxidant studies

The antioxidant activity of the pineapple crude juice was assessed by studying its effect on scavenging nitric oxide (NO·) and α,α$_2$-diphenyl-β-picrylhydrazyl (DPPH·) radicals according to Garrat (1964), Liyana-Pathiranan and Shahidi (2005)respectively. The reducing potential of the pineapple juice was determined by ferric reducing antioxidant power (FRAP) test (Oyaizu, 1986).

Ascorbic acid (200µg/ml) and tert-butylhydroquinone (TBHQ; 600 and 150µg/ml) were used as reference standards, respectively. The capacity of the juice for iron chelation was measured by the method of Tang *et al.,* (2002) using ethylenediamintetra acetic acid (EDTA; 100µg/ml) as standard. Inhibition of lipid peroxidation was also determined using freshly prepared 25% rat liver homogenate as a source of polyunsaturated fatty acids. The percentage of inhibition of lipid peroxidation of the juice was assessed by comparing the absorbance of the reaction mixture containing the juice with that of control (The reaction mixture without the juice) (Liu and Ng, 2002).

Animals

A total of 32 adult male albino rats weighing 180-200g obtained from the breeding unit of the Medical Research Center (Faculty of Medicine, Ain Shams University, Cairo) were used throughout this study. The rats were housed in steel mesh cages (2/cage) on wood-chip bedding and maintained on a commercial pellet diet and tap water for one week before the start of the experiment as an acclimatization period. All animal experiments were performed according to the protocols approved by the local institutional animal ethics committee of Ain Shams University.

Induction of hypothyroidism

Experimental hypothyroidism was induced in rats by oral administration of methimazole (MW 114.17) (Sigma Aldrich chemical Co., USA) in drinking water (0.02%w/v) for 15 days (Venditti *et al.*, 1997).

Biochemical studies

Study design

The animals were randomly divided into four equally sized groups as follows: Group I. normal control (euthyroid) (NC), normal euthyroid rats were fed a commercial pellet diet and tap water; Group II. pineapple juice (PJ), normal rats were daily supplemented with undiluted whole pineapple juice, Group III. hypothyroidism (Hypo), rats were intoxicated with methimazole and Group IV. (Hypo+PJ), rats were first intoxicated with methimazole, then supplemented with the pineapple juice.

Blood collection and tissue sampling

At the end of the experiment, the animals were weighed and dissected under light ether anesthesia after a fast of 12 hours. Blood samples were withdrawn from the abdominal aorta and serum was separated from the clotted blood samples by centrifugation at 5,000 rpm for 5 min then aliquoted. The thyroid glands, liver and heart were dissected out and rinsed in sterile saline. The whole thyroid glands were preserved in 10% phosphate buffered formalin (pH 7.2) at 4°C for 3 days before histological examination. The heart and liver tissues were stored in sterile saline at -80°C until biochemical analyses.

Preparation of tissue homogenates

Heart and liver tissues were weighed then homogenized in ice-cold phosphate buffered saline (pH 7.4) to prepare 10% (w/v) whole tissue homogenate. Aliquots of the whole tissue homogenates were centrifuged at 10,000 rpm for 15 min at 4°C to obtain the cytosolic supernatants. The whole tissue homogenates and the cytosolic supernatants were preserved at-20°C until biochemical analyses.

Biochemical assays

Serum total T4 and total T3 levels were determined using a research assay ELISA Kit for rat (EIAab, China). Serum triacylglycerols (TG) and high density lipoprotein-cholesterol (HDL-C) levels were determined colorimetrically using commercial assay kits (Biovision, USA). Serum total and free cholesterol (TC & FC) levels were determined according to the method of Zak *et al.*(1954). Serum cholesteryl esters (EC) level was calculated by subtracting free cholesterol (FC) from total cholesterol. Percentage of cholesterol esterification was calculated from the formula (%= EC (mg/dl)/TC (mg/dl)×100). Serum low density lipoprotein-cholesterol (LDL-C) and very low density lipoprotein-cholesterol (VLDL-C) levels were also calculated (Friedewald *et al.*, 1972). Atherogenic index (AI) was calculated from the formula (AI=TC-HDL-C/HDL-C) (Wilson *et al.*, 1980). Lipid peroxides were assessed colorimetrically in serum, whole heart and liver homogenates as thiobarbituric acid-MDA adduct concentration using a commercial assay kit (Biovision, USA).

Enzymes assays

Serum lecithin cholesterol acyltransferase (LCAT, EC 2.3.1.43) activity was determined using a research ELISA Kit (EIAab, China). The activity of glutathione peroxidase (GPx, EC 1.11.1.9) was also determined in serum and the cytosolic supernatants of heart and liver homogenates using a commercial assay kit (Biovision, USA). Total protein concentration was determined in the same cytosolic supernatants according to Lowry *et al.* (1951), and the enzyme activities were expressed as units per milligram protein.

Histological studies

Fixed thyroid gland specimens were furtherly processed to form paraffin cubes, then thin sections of 4µm thickness were stained with hematoxylin and eosin (H & E). The slides were examined and photographed under a light microscope at a magnification power of (x 160).

Statistical analysis

Results were expressed as means ± SD and statistically analyzed using one way analysis of variance (ANOVA). In case of significance, posthoc Bonferroni test for multiple comparisons was done using SPSS (version 14.0) (Chicago, USA). Differences were considered significant at ρ value less than 0.05.

Results

In vitro antioxidant experiments showed that the pineapple juice had a high scavenging activity for $NO^{\cdot}$ (207%), and $DPPH^{\cdot}$ radicals (700%), compared to their respective reference standards; ascorbic acid (45.4%) and tertiary-butylhydroquinone (87%), respectively. Additionally, the whole pineapple juice exhibited a drastic inhibitory effect on the production of thiobarbituric reactive substances (489%) and a high ferric reducing antioxidant power (240%), whereas it showed no iron chelating activity (Table 1).

Table 1. The activity of pineapple juice for scavenging NO and $DPPH^{\cdot}$ radicals, iron chelation, FRAP and inhibition of lipid peroxidation

Parameters	Pineapple juice	Standards			
		Vit C	EDTA	TBHQ	TBHQ
Conc.	100ml	(200µg/ml)	(100µg/ml)	(600µg/ml)	(150µg/ml)
NO Scavenging activity (%)	207	45.4	_	_	_
$DPPH^{\cdot}$ Scavenging activity (%)	700	_	_	87	_
Fe^{2+} chelating activity (%)	0	_	100	_	_
FRAP	240	_	_	_	100
Inhibition of lipid peroxidation (%)	489	_	_	_	_

DPPH, α,α-diphenyl-β-picrylhydrazyl; EDTA, ethylenediaminetetra acetic acid; FRAP, ferric reducing antioxidant power; NO, nitric oxide; TBHQ, tert-butylhydroquinone; Vit. C, vitamin C. * Experiments were carried out in triplicates.

Daily supplementation of euthyroid rats with the pineapple juice produced a significant reduction in the body weight (16.80%) and a significant increase in serum T_3 and T_4 levels61.10 and 22.20%, respectively), compared to normal controls (Table 2). Administration of MMI to rats in drinking water (0.02%w/v) for 15 days induced hypothyroidism manifested by significant reductions in serum levels of T_3 and T_4 (34.20 and 46.60%, respectively) associated with a significant increase in the body weight (52.20%), as well as degenerative changes in the structure of the thyroid gland follicles, compared to normal controls (Table 2 & Fig. 3). Supplementation of whole pineapple juice to hypothyroid rats substantially improved the thyroid gland function as evident by normalization of the body weight and serum T_3 level, while serum T_4 level was persistently reduced (42.20%), compared to normal controls (Table 2).

Table 2. Effect of pineapple juice on body weight and serum T_3 and T_4 levels in all studied groups.

Parameters	Groups			
	GI (NC)	GII (PJ)	GIII (Hypo)	GIV (Hypo+PJ)
Initial BW(g)	217±13.60 [a]	210±9.92 [a]	225±14.40 [a]	218±13.09 [a]
change% from NC		- 3.23	3.69	0.46
change% from Hypo				- 3.11
Final BW(g)	248±12.17 [a]	235±12.39 [a]	274±15.08 [b]	250±14.56 [a]
change% from NC		- 5.24	10.08	6.45
change% from Hypo				- 8.76
BW gain (%)	14.3±2.67 [a]	11.9±1.72 [b]	21.8±1.82 [c]	14.7±2.70 [a]
change% from NC		- 16.8	52.5	2.8
change% from Hypo				- 47.3
T_3(ng/dl)	88.4±6.04 [a]	142.5±7.08 [b]	58.2±4.98 [c]	78.8±9.77 [a]
change% from NC		61.1	- 34.2	- 10.9
change% from Hypo				35.4
T_4(μg/dl)	4.5±0.48 [a]	5.5±0.44 [b]	2.4±0.36 [c]	2.6±0.35 [c]
change% from NC		22.2	- 46.6	- 42.2
change% from Hypo				8.3

Values are represented as mean ± SD of 8 rats. Each value is considered statistically significant at $\rho<0.05$. Groups sharing the same superscripts are not statistically different.

Supplementation of euthyroid rats with the pineapple juice produced insignificant changes in the serum lipid profile and atherogenic index, compared to normal controls. On the other hand, hypothyroid rats manifested significant elevations in serum levels of triacylglycerols (68.89%), total cholesterol (43.46%), VLDL-C (68.89%), LDL-C (84.36%) and atherogenic index (AI) (68.92%), compared to normal controls (Table 3). In comparison of hypothyroid-supplemented rats to the hypothyroid group, highly significant reduction in serum levels of triacylglycerol (29.30%), total cholesterol (22.56%), VLDL-C (29.30%), LDL-C (39.59%) and atherogenic index (36.80%) is reported (Table 3).

Table 3. Effect of pineapple juice on serum LCAT activity and lipid profile in all studied groups

Parameters	Groups			
	G I (NC)	GII (PJ)	GIII (Hypo)	GIV(Hypo+PJ)
LCAT (U/l)	1.89±0.19 [a]	2.05±0.14 [a]	1.42±0.16 [b]	1.77±0.13 [a]
change% from NC		8.47	- 24.87	-6.35
change% from Hypo				24.6
Triacylglycerol (mg/dl)	67.5±5.40 [a]	71.4±7.20 [a]	114±10.10 [b]	80.6±10.60 [c]
change% from NC		5.78	68.89	19.41
change% from Hypo				- 29.30
Total Cholesterol (mg/dl)	81.0±7.03 [a]	84.46±5.53 [a]	116.2±4.01 [b]	90.0±6.53 [b]
change% from NC		4.27	43.46	11.11
change% from Hypo				- 22.56
Free Cholesterol (mg/dl)	18.82±1.57 [a]	19.91±1.93 [a]	39.98±3.45 [b]	20.56±1.61 [a]
change% from NC		5.79	112.43	9.25
change% from Hypo				- 48.57
Cholesteryl esters (mg/dl)	62.18±3.68 [a]	65.05±6.51 [a]	76.22±5.11 [b]	69.44±3.97 [c]
change% from NC		4.62	22.58	11.68
change% from Hypo				- 8.90
% of Esterification	76.8 [a]	77.02 [a]	65.59 [b]	77.16 [a]
change% from NC		0.29	-14.60	0.47
change% from Hypo				17.64

VLDL-C (mg/dl)	13.5±1.04[a]	14.28±1.43[a]	22.8±2.03[b]	16.12±2.11[c]
change% from NC		5.78	68.89	19.41
change% from Hypo				- 29.30
LDL-C (mg/dl)	21.1±2.0[a]	21.38±3.02[a]	38.9±3.84[b]	23.5±2.57[a]
change% from NC		1.33	84.36	11.37
change% from Hypo				- 39.59
HDL-C (mg/dl)	46.4±4.19[a]	48.8±7.69[a]	49.5±5.28[a]	50.38±5.35[a]
change% from NC		5.17	6.68	8.58
change% from Hypo				1.8
AI	0.74±0.06	0.73±0.07[a]	1.25±0.05[b]	0.79±0.08[a]
change% from NC		- 1.35	68.92	6.76
change% from Hypo				- 36.8

Values are represented as mean ± SD of 8 rats. Each value is considered statistically significant at ρ<0.05. Groups sharing the same superscripts are not statistically different.

In the present study euthyroid rats supplemented with the pineapple juice showed insignificant changes in serum levels of Lecithin cholesterol acyltransferase (LCAT) activity, free cholesterol and cholesteryl esters, compared to normal controls. However, hypothyroid rats manifested significant reduction in serum levels of LCAT activity (24.87%) and the percentage of cholesterol esterification(14.60%),and in contrast significant elevation in serum levels of free cholesterol (112.43%) and cholesteryl esters(22.58%), compared to normal controls (Table 3).The reduced LCAT activity and enhanced hyperlipidemia observed in the hypothyroidism group were substantially ameliorated by the pineapple juice supplementation, whereby significant elevation in serum levels of LCAT activity (24.60%) and the percentage of cholesterol esterification(17.64%),and in contrast significant reduction in free cholesterol (48.57%) and cholesteryl esters (8.90%) levels were recorded, compared to the hypothyroid group (Table 3).

Subchronic supplementation of euthyroid rats with the whole pineapple juice produced insignificant changes in serum, hepatic and cardiac GPx activity and MDA concentration, compared to the normal controls. Induction of experimental hypothyroidism significantly decreased serum, hepatic and cardiac GPx activity (36.87, 34.84 and 33.92%, respectively), and in contrast significantly elevated serum, hepatic and cardiac MDA concentrations (66.67, 50.61 and 38.67%, respectively), compared to normal controls. In hypothyroid-supplemented group (Gr IV), serum, hepatic and cardiac GPx activity were significantly increased (47.22, 45.13 and 47.14%, respectively), while serum, hepatic and cardiac MDA levels were significantly decreased (35.73, 31.85 and 26.45%,respectively), compared to the hypothyroid group, and returning them back to the normal values (Table 4).

Table 4. Effect of pineapple juice on serum, hepatic and cardiac GPX activity and MDA concentration in all studied groups.

Parameters	Groups			
GPX	GI (NC)	GII (PJ)	GIII (Hypo)	GIV (Hypo+PJ)
Sreum (U/ml)	66.32±4.64a	70.11±5.60[a]	41.87±4.90[b]	61.64±5.4[a]
change% from NC		5.71	- 36.87	- 7.06
change% from Hypo				47.22
Liver (U/ml)	31.52±2.93[a]	30.38±3.13[a]	20.54±2.48[b]	29.81±2.88[a]
change% from NC		- 3.28	- 34.84	- 5.43
change% from Hypo				45.13
Heart (U/ml)	25.47±2.92[a]	25.13±3.19[a]	16.83±1.41[b]	24.75±1.86[a]
change% from NC		- 1.34	- 33.92	- 2.83
change% from Hypo				47.14
MDA				
Sreum (nmol/ml)	2.25±0.26[a]	1.98±0.22[a]	3.75±0.29[b]	2.41±0.31[a]
change% from NC		- 12	66.67	7.11
change% from Hypo				- 35.73
Liver (nmol/g tissue)	42.76±2.97[a]	41.82±1.40[a]	64.40±5.49[b]	43.89±4.84[a]
change% from NC		- 2.20	50.61	2.64

change% from Hypo				- 31.85
Heart (nmol/g tissue)	36.72±4.93[a]	37.31±4.36[a]	50.92±4.35[b]	37.45±4.48[a]
change% from NC		1.61	38.67	2.0
change% from Hypo				- 26.45

Values are represented as mean ± SD of 8 rats. Each value is considered statistically significant at $\rho<0.05$.Groups sharing the same superscripts are not statistically different.

Histological examination of the thyroid tissue sections from normal controls and pineapple supplemented rats that were stained with hematoxylin and eosin showed large round thyroid follicles filled with pale colloid. Thyrocytes were cuboidal or somewhat flattened. Capillaries in the connective tissue among the follicles were narrowed (Fig.1&2).

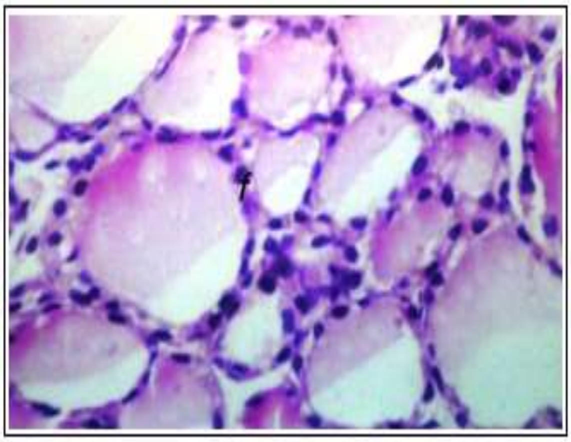

Fig. 1. Thyroid tissue section of a normal rat showsthyroid follicles lined with cuboidal epithelium with eosinophilic colloid (C) in the lumen (x 160).

Thyroid tissue sections from MMI-intoxicated rats showed degenerative changes with different types of follicles and empty follicular lumen. The majority of the follicles were irregularly shaped and narrowed due to resorbed colloid. The thyrocytes were enlarged and more or less cylindrical in shape (Fig. 3).

Conversely, the thyroid follicles of pineapple juice-supplemented hypothyroid rats were lined with active cuboidal epithelial cells, which revealed an improvement in the cellular architecture of the thyroid gland epithelium lining the follicles, while the follicular lumen was partially filled with colloid (Fig. 4).

Discussion

Antioxidant activity of pineapple juice while allopathic treatment for hypothyroidism involves medication with T4, alternative treatment focuses on supporting the thyroid function by supplying nutrients necessary for the synthesis of the thyroid hormone, as well as for enhancing cellular conversion of T4 to T3. In the present study, the effect of subchronic supplementation with the crude pineapple juice from the whole fruit on experimental hypothyroidism was investigated. The pineapple juice is characterized by its high content of vitamin C and the proteolytic enzyme; bromelain, in addition to the phenolic compounds, such as p-coumaric acid, caffeic acid, ferulic acid, sinapic acid, p-coumaroylquinic acid, feruloylglucose, p-hydroxybenzoic acid, p-hydroxybenzoic aldehyde and syringic acid (Wen and Worlsted, 2002). The unique composition of the pineapple juice probably accounts for its high antioxidant activity, exemplified by the high scavenging potential for NO and DPPH· radicals, as well as the drastic inhibitory effect of lipid peroxidation at the implemented conditions, compared to their reference standards.

Effect of pineapple juice on body weight and serum T3 and T4 levels

MMI was chosen to induce hypothyroidism in the rats, as it is easily absorbed by the gastrointestinal tract and concentrated in the thyroid gland (Aboul-Enein and Al-Badr, 1979). It produces hypothyroidism by inhibiting the production of thyroid hormone (Aletrari *et al.,* 1998). MMI acts as a false substrate for thyroid peroxidase, thus inhibiting the incorporation of iodide into tyrosine residues on thyroglobulin catalyzed by thyroid peroxidase (Bandyopadhyay *et al.*, 2002). Although the detailed mechanism of its action is still not clear, yet the available information *reveals* that this drug may block the thyroid hormone synthesis by coordinating to the metal center of the thyroid peroxidase (Roy *et al*., 2007).

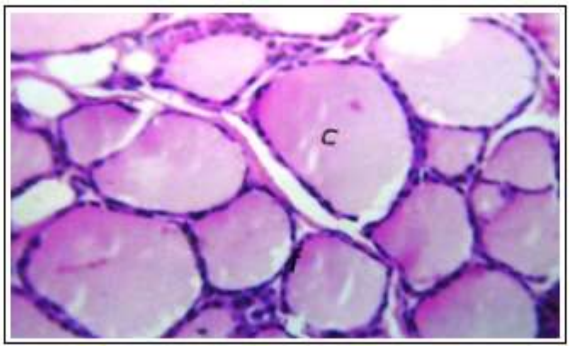

Fig. 2. Thyroid tissue section of a normal rat supplemented with the pineapple juice shows a flattened epithelium lining the follicles with eosinophilic colloid (C) in the lumen (x160).

Administration of MMI to rats in drinking water (0.02%w/v) for 15 days induced hypothyroidism manifested by a significant increase in the body weight and significant decrease in serum thyroid hormone levels, as well as degenerative changes in the structure of the thyroid gland follicles.

Supplementation of whole pineapple juice to hypothyroid rats for 8 weeks substantially improved the thyroid gland functions as evident by the normalization of the body weight and serum T3 level, while serum T4 level was persistently reduced. The biochemical findings were confirmed by the histological examination of H&E-stained thyroid tissue sections, which revealed an improvement in the cellular architecture of thyroid gland epithelium lining the follicles and the colloid content of the follicular lumen. The increase in serum T3 level at the expense of T4 level might be due to the stimulatory effect of the pineapple juice on the enzymes responsible for the peripheral conversion of T4 to T3 or on the iodination process of T3 to T4. However, these explanations need further investigation. Previous studies conducted on some of the components of the pineapple juice reported parallel results. Gupta and Kar (1998), have shown that treatment of normal rats with vitamin C (1mM, equivalent to human therapeutic dose) has no effect on the thyroid hormone levels, the antioxidant enzyme systems, and lipid peroxidation; however it partially restores the thyroid gland functions to normal in rats intoxicated with cadmium. Also, Ibtissem *et al.,* (2009) have demonstrated that MMI-induced hypothyroidism in rats could be prevented by dietary supplementation with selenium (0.5mg sodium selenite/Kg diet), and that this trace element could be of great importance to health since it activates the thyroid hormones synthesis.

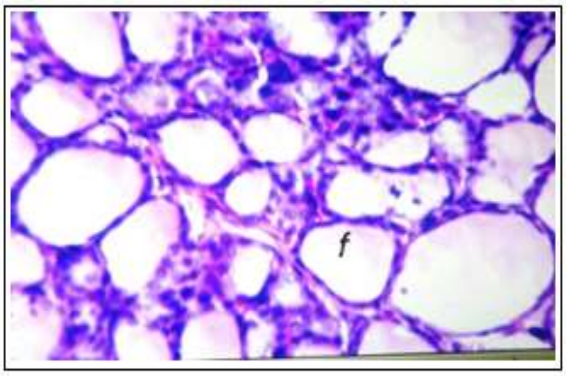

Fig. 3. Thyroid tissue section of hypothyroid rat shows degeneration and vacuolization in the cytoplasm of the thyroid follicules (f) and empty follicular lumen (x160).

Effect of pineapple juice on serum LCAT activity and lipid profile

Chronic hypothyroidism is frequently associated with atherosclerosis due to increased plasma cholesterol levels and the contribution of impaired reverse cholesterol transport (RCT). Measurement of plasma HDL-C level alone is not an appropriate method to assess the dynamic process of cholesterol transfer from the peripheral tissues to the liver, neither is a good indicator of the efficiency of RCT system in the prevention of atherosclerosis. Instead, the best indicator of HDL metabolism and its role in RCT system is the related enzyme, namely serum lecithin cholesterol acyltransferase (LCAT) activity (Franco *et al.,* 2003). LCAT, synthesized mainly in liver, is a key enzyme for the production of cholesteryl esters in the plasma and it promotes the formation of high density lipoprotein (HDL). LCAT was proposed to promote the RCT, the anti-atherogenic mechanism by which excess cholesterol is removed from cells by HDL and delivered to the liver for excretion.

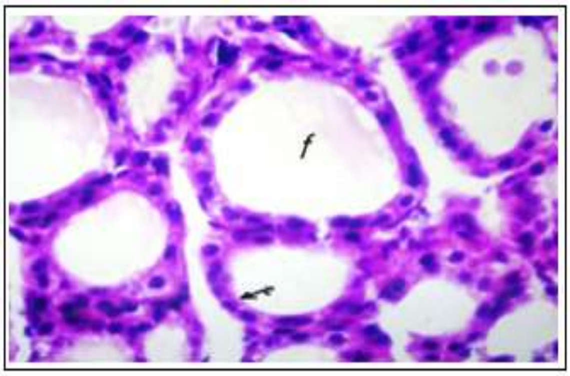

Fig. 4. Thyroid tissue section of hypothyroid rats supplemented with the pineapple juice shows intact lining of active cuboidal epithelium, while the follicular lumen was still partially empty (x160).

In the present study MMI-induced hypothyroidism produced significant reduction in serum level of LCAT activity along with the percentage of cholesterol esterification, and conversely significant elevations in serum levels of TC, TG, LDL-C and AI. Our findings are in accordance with those of Ridgway and Dolphin (1985), who attributed the reduced serum LCAT activity due to depressed hepatic secretion. The reduced LCAT activity and enhanced hyperlipidemia observed in the hypothyroidism group were substantially ameliorated by the pineapple juice supplementation.

The anti-hyperlipidemic effect of the pineapple juice might be due to its high content of vitamin C, which activates the enzyme 7α-hydroxylase and enhances the conversion of plasma cholesterol to bile acids, hence resulted in reduction of serum cholesterol level. Our findings are more pronounced than those of Eteng *et al.,* (2006), who affirmed that oral administration of vitamin C (200 mg/kg body weight) significantly decreases serum total cholesterol (16.34%), VLDL-C (21.15%) and LDL-C (8.7%) concentrations.

The augmentation might be due to the longer treatment period in the present study, and to the presence of polyphenol compounds, which have been previously shown to inhibit cholesterol absorption and biosynthesis and to promote the expression of LDL-C receptors (Jia *et al.,* 2010). Mondal *et al.*, (2011) reported that rats consuming pineapple juice over a three-hour period experience a decrease in lipoprotein particles and increased metabolism; activities that lower cholesterol levels.

Effect of pineapple juice on serum, hepatic and cardiac GPX activity and MDA concentration

Glutathione peroxidase as one of the most important antioxidant enzymes, and malondialdehyde, as a byproduct of peroxidation of biologic membrane polyunsaturated fatty acids, were measured in the serum, liver and heart to reflect the status of the antioxidant defense system, and as an indicator of the oxidative stress, respectively, after pineapple juice supplementation.

The significant decline in GPX activity associated with the significant elevation in serum, hepatic and cardiac MDA concentrations in the hypothyroid rats reflect the severe oxidative stress induced by MMI intoxication. Parallel results were previously reported (Moulakakisi *et al.,* 2008).

Additionally, the sharp increase in the body weight of the hypothyroid rats was previously reported and explained due to enhanced production of reactive oxygen species (ROS), which upregulate the expression of NADPH oxidase establishing a vicious cycle that augments the oxidative stress in the adipocytes and blood circulation (Furukawa *et al.,* 2004).

Supplementation of hypothyroid rats with the whole pineapple juice normalized serum, hepatic and cardiac MDA concentrations, as well as GPX activities. These results are in agreement with those of Aiyegbusi *et al.*, (2011),who reported that the pineapple juice significantly lowers the MDA level, compared with normal controls and pineapple juice treated rats.

These findings could be interpreted due to the high antioxidant activity and the ferric reducing antioxidant power of the pineapple juice from the whole fruit, which act collectively to reduce the production of ROS by inhibiting lipid peroxidation and enhancing the scavenge of the free radicals, which are known to be increased in the hypothyroidism.

Conclusion

In conclusion, supplementation of the whole pineapple juice has shown a strong antioxidant activity, which plays a central role in activating the thyroid hormones synthesis in hypothyroid rats, as well as anticipating the hyperlipidemia associated with hypothyroidism, which is a risk factor for coronary heart diseases.

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